

THE
AMERICAN JOURNAL
OF
PHYSIOLOGY

VOLUME LIII

BALTIMORE, MD.
1920



AUG 18 1920

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	PAGE
THE TRANSFUSION EXPERIMENT WITH RED BLOOD CORPUSCLES. <i>Hisanobu Kambe and Etsuzo Komiya</i>	1
ON THE REGENERATION OF THE VAGUS NERVE. <i>F. T. Rogers</i>	15
EFFECT OF VARIOUS SUBSTANCES UPON THE COAGULATION OF CITRATED PLASMA. <i>Ben Karpman</i>	25
THE INFLUENCE OF PITUITARY EXTRACTS ON THE ABSORPTION OF WATER FROM THE SMALL INTESTINE. <i>Maurice H. Rees</i>	43
THE SPECIFIC INFLUENCE OF THE ACCELERATOR NERVES ON THE DURATION OF VENTRICULAR SYSTOLE. <i>Carl J. Wiggers and Louis N. Katz</i>	40
GASTRIC RESPONSE TO FOODS. XIII. THE INFLUENCE OF SUGARS AND CANDIES ON GASTRIC SECRETION. <i>Raymond J. Miller, Olaf Bergeim, Martin E. Rehfsuss and Philip B. Hawk</i>	65
FURTHER EVIDENCE ON THE FUNCTIONAL CORRELATION OF THE HYPOPHYSIS AND THE THYROID. <i>John A. Larson</i>	89
THE INFLUENCE OF AN ALCOHOLIC EXTRACT OF THE THYROID GLAND UPON POLYNEURITIC PIGEONS AND THE METAMORPHOSIS OF TADPOLES. <i>Emily C. Seaman</i>	101
THE ALKALI RESERVE IN EXPERIMENTAL SURGICAL SHOCK. <i>Bernard Raymund</i>	109
PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE. III. THE PRODUCTION OF LIGHT BY LUCIOLA VITICOLLIS IS AN OXIDATION. <i>Sakyo Kanda</i>	137

VOL. LIII—No. 1

Issued August 1, 1920

BALTIMORE, U. S. A.

1920

THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From two to three volumes each of about five hundred pages are published yearly. The subscription price per volume in the United States and Canada is \$5.00; in other countries \$5.25.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, Johns Hopkins Medical School, Washington and Monument Streets, Baltimore, Md. They should be packed flat, carefully protected from injury and insured.

Each article should conclude with a brief summary of results suited to the needs of reference journals.

References to literature cited should contain: first the name of the author or authors, second the name of the journal or book; third the year; fourth the volume number and fifth the page. These references should be collected on a separate page at the end of the article with corresponding bracketed numbers in the text. Foot notes are to be avoided whenever possible.

All figures for illustration should be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters to figures cannot be set in type and should, therefore, be written in India ink and regarded as a part of the figure. When artist's work is required to make figures suitable for reproduction this will be charged to authors. Unless specific instructions are given by authors the printer will be allowed to use his judgment in determining the amount of reduction on all figures. In any case the plates should never exceed the dimensions of the printed matter of the JOURNAL page unless the author is prepared to pay the cost of an insert page.

Tracings on smoked paper reproduce well if the white lines are distinct and separate and the background uniform black. Authors may safely retouch breaks or weak spots in the white lines with white ink and fill in the background with India ink. Curves laid on coordinate paper should be in India ink. Background lines in blue will not reproduce; red, yellow and green will reproduce black. Plotted curves and drawings yield better results if the originals are large enough to permit of considerable reduction in the plates used for illustration, but authors are warned to make due allowance for this in the breadth of lines and size of lettering. Particular letters and numbers to paste on tracings and figures will be furnished upon request if the amount of reduction to be used is stated.





CONTENTS

No. 1. AUGUST 1, 1920

THE TRANSFUSION EXPERIMENT WITH RED BLOOD CORPUSCLES. <i>Hisanobu Kambe and Etsuzo Komiya</i>	1
ON THE REGENERATION OF THE VAGUS NERVE. <i>F. T. Rogers</i>	15
EFFECT OF VARIOUS SUBSTANCES UPON THE COAGULATION OF CITRATED PLASMA. <i>Ben Karpman</i>	25
THE INFLUENCE OF PITUITARY EXTRACTS ON THE ABSORPTION OF WATER FROM THE SMALL INTESTINE. <i>Maurice H. Rees</i>	43
THE SPECIFIC INFLUENCE OF THE ACCELERATOR NERVES ON THE DURATION OF VENTRICULAR SYSTOLE. <i>Carl J. Wiggers and Louis N. Katz</i>	49
GASTRIC RESPONSE TO FOODS. XIII. THE INFLUENCE OF SUGARS AND CANDIES ON GASTRIC SECRETION. <i>Raymond J. Miller, Olaf Bergeim, Martin E. Rehfuess and Philip B. Hawk</i>	65
FURTHER EVIDENCE ON THE FUNCTIONAL CORRELATION OF THE HYPOPHYSIS AND THE THYROID. <i>John A. Larson</i>	89
THE INFLUENCE OF AN ALCOHOLIC EXTRACT OF THE THYROID GLAND UPON POLYNEURITIC PIGEONS AND THE METAMORPHOSIS OF TADPOLES. <i>Emily C. Seaman</i>	101
THE ALKALI RESERVE IN EXPERIMENTAL SURGICAL SHOCK. <i>Bernard Raymund</i>	109
PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE. III. THE PRODUCTION OF LIGHT BY <i>LUCIOLA VITICOLLIS</i> IS AN OXIDATION. <i>Sakyo Kanda</i> ...	137

No. 2. SEPTEMBER 1, 1920

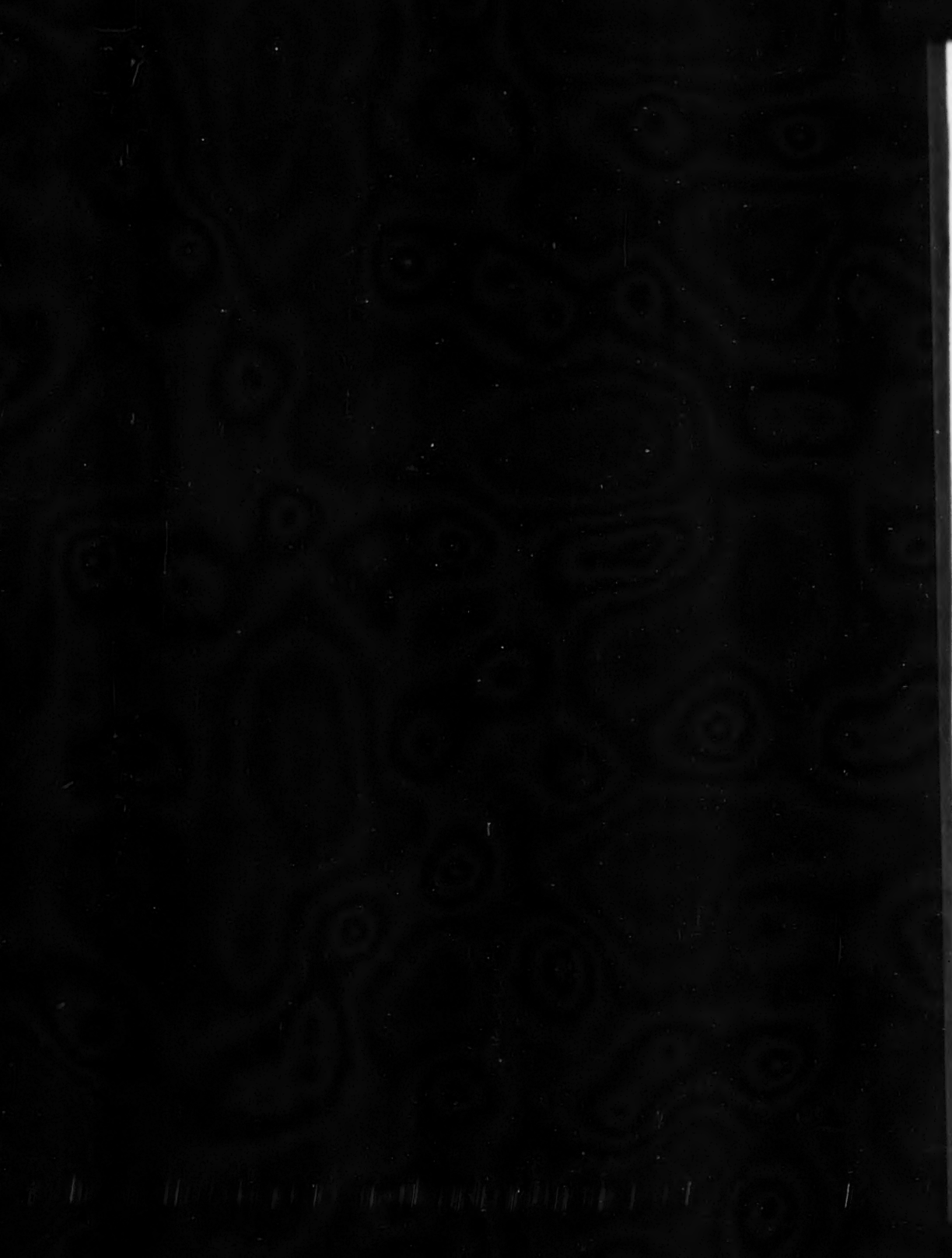
BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA. I. MIXED DIET REACTION. <i>G. H. Whipple, C. W. Hooper, and F. S. Robscheit</i>	151
BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA. II. FASTING COMPARED WITH SUGAR FEEDING. ANALYSIS OF "SPARING ACTION OF CARBOHYDRATES." <i>G. H. Whipple, C. W. Hooper and F. S. Robscheit</i> ..	167
BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA. III. INFLUENCE OF BREAD AND MILK, CRACKERMEAL, RICE AND POTATO, CASEIN AND GLIADIN IN VARYING AMOUNTS AND COMBINATIONS. <i>C. W. Hooper, F. S. Robscheit and G. H. Whipple</i>	206
BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA. IV. INFLUENCE OF MEAT, LIVER AND VARIOUS EXTRACTIVES, ALONE OR COMBINED WITH STANDARD DIETS. <i>G. H. Whipple, F. S. Robscheit and C. W. Hooper</i> ..	236
BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA. V. THE INFLUENCE OF BLAUD'S PILLS AND HEMOGLOBIN. <i>C. W. Hooper, F. S. Robscheit and G. H. Whipple</i>	263

PHYSIOLOGIC CHANGES PRODUCED BY VARIATIONS IN LUNG DISTENTION.	
III. IMPAIRMENT OF THE CORONARY CIRCULATION OF THE RIGHT VENTRICLE. <i>Ralph Hopkins and Felix P. Chillingworth</i>	283
VAGUS AND SPLANCHNIC INFLUENCE ON THE GASTRIC HUNGER MOVEMENTS OF THE FROG. COMPARATIVE STUDIES III. <i>T. L. Patterson</i>	293
OBSERVATIONS ON THE RELATION BETWEEN EMOTIONAL AND METABOLIC STABILITY. <i>Frederick S. Hammelt</i>	307
FOUR FACTORS CAUSING CHANGES IN THE TYPE OF RESPONSE OF THE ISOLATED INTESTINAL SEGMENT OF THE ALBINO RAT (<i>MUS NORVEGICUS ALBINUS</i>) TO SODIUM CARBONATE. <i>S. Hatai and F. S. Hammelt</i>	312
THE ADJUSTMENT OF BLOOD VOLUME AFTER INJECTION OF ISOTONIC SOLUTIONS OF VARIED COMPOSITION. <i>Arthur H. Smith and Lafayette B. Mendel</i>	323

No. 3. OCTOBER 1, 1920

ANTAGONISM OF INHIBITORY ACTION OF ADRENALIN AND DEPRESSION OF CARDIAC VAGUS BY A CONSTITUENT OF CERTAIN TISSUE EXTRACTS. <i>J. B. Collip</i>	343
RECIPROCAL REACTIONS IN THE CARDIO-VASCULAR SYSTEM. <i>Ethel W. Wickwire</i>	355
FURTHER OBSERVATIONS ON THE RELATION OF INITIAL LENGTH AND INITIAL TENSION OF AURICULAR FIBER ON MYO- AND CARDIODYNAMICS. <i>Robert Gesell</i>	377
THE RÔLE OF THE PANCREAS IN HYPERGLYCEMIA FROM ETHER. <i>Ellison L. Ross and L. H. Davis</i>	391
PHYSIOLOGICAL STUDIES ON PLANARIA. IV. A FURTHER STUDY OF OXYGEN CONSUMPTION DURING STARVATION. <i>Libbie H. Hyman</i>	399
VASOMOTOR REFLEXES FROM RECEPTOR STIMULATION IN INTACT ANIMALS. <i>E. G. Martin, A. C. Franklin and Clarence Held</i>	421
STUDIES IN PLACENTAL PERMEABILITY. I. THE DIFFERENTIAL RESISTANCE TO CERTAIN SOLUTIONS OFFERED BY THE PLACENTA IN THE CAT. <i>R. S. Cunningham</i>	439
THE PRODUCTION OF INTRACELLULAR ACIDITY BY NEUTRAL AND ALKALINE SOLUTIONS CONTAINING CARBON DIOXIDE. <i>M. H. Jacobs</i>	457
THE EFFECT OF SALT INGESTION ON CEREBRO-SPINAL FLUID PRESSURE AND BRAIN VOLUME. <i>Frederic E. B. Foley and Tracy Jackson Putnam</i>	464
ANTAGONISM OF DEPRESSOR ACTION OF SMALL DOSES OF ADRENALIN BY TISSUE EXTRACTS. <i>J. B. Collip</i>	477
OBSERVATIONS ON A SEX DIFFERENCE IN THE PRESENCE OF NATURAL HEMOLYSIN IN THE RAT. <i>Yoshio Suzuki</i>	483
STUDIES ON ABSORPTION FROM SEROUS CAVITIES. III. THE EFFECT OF DEXTROSE UPON THE PERITONEAL MESOTHELIUM. <i>R. S. Cunningham</i>	488
INDEX.....	495





THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 53

AUGUST 1, 1920

No. 1

THE TRANSFUSION EXPERIMENT WITH RED BLOOD CORPUSCLES

HISANOBU KAMBE AND ETSUZO KOMIYA

From the Medical Department, the Imperial University, Tokyo, Japan

Received for publication, April 6, 1920

What is the fate of red blood corpuscles if they have been introduced into an animal body of same species? This problem has been studied by numerous investigators (1), (2), (3) and though there are still objections it is generally believed that the transfused red cells are capable of functioning in the animal body. Whether these red cells remain and function in circulation of a recipient animal or whether they are decomposed, was formerly studied chiefly by means of the examination of the changes of urine or the bodily condition of the animal. Recent opinion indicates that the most satisfactory method of attacking the problem as to the length of life of the transfused corpuscles is to study the changes in erythrocyte count following transfusion.

In the earlier work in which this method was used little attention was given to morphological changes in the corpuscles. This is a point of considerable importance because from the erythrocyte count alone we are not justified in drawing conclusions as to the biological phenomena exhibited by the foreign corpuscle or its host. It is therefore desirable to collect more data on the subject.

EXPERIMENTAL

In the present experiments full-grown rabbits were used. To obtain blood from a donor the carotid artery was exposed by usual method and opened on one side with a sterile scalpel. The blood thus collected in a sterile, thick-walled flask containing glass beads, was defibrinated by vigorous shaking, filtered into another sterile flask through double

gauze, and preserved for injection. Sometimes blood was kept in a mixture of isotonic citrate and dextrose solution, and when this mixture was used the supernatant fluid was pipetted away.

To render a recipient animal anemic, blood q. l. was withdrawn from carotid artery without anesthesia, care being taken to avoid the shock which may be caused by a rapid hemorrhage. The blood obtained from the donor was then transfused into the ear vein of recipient rabbits using a sterile syringe. At the time of injection the blood was warmed to the body temperature. In each series of experiments, the number of red cells, hemoglobin content and morphology of the blood were carefully studied. The number of red cells was counted by the Thoma-Zeiss apparatus, and hemoglobin content was estimated by Sahli's hemometer. Blood cells were stained in May-Grünwald-Giemsa's solution. Each experiment was repeated several times with almost the same result. To avoid a repetition of data only one instance for each case is recorded here.

Control experiment. In the first place an experiment was done to observe in what manner the natural restoration of the anemia caused by hemorrhage takes place and what changes in the direction of morphology are brought about.

Experiment 1. Rabbit weighing 2500 grams was bled 65 cc. from the carotid artery. The animal has been always in a good condition.

It may be seen from figure 1 that if the blood of 20-30 cc. per kilogram of body weight is depleted, the number of red cells of the animal is generally reduced by 40 per cent or more of its original amount and with a gradual improvement it will return to normal level two weeks later.

As to morphological changes, polychromatophilia was especially noticeable. This kind of red cells appeared in twelve or twenty-four hours after hemorrhage, and gradually increasing in number they reached the highest point on the third or fourth day, disappearing again in the course of a week. These polychromatic red cells are larger than normal red cells, so the blood has appearance of a remarkable anisocytosis. Erythroblasts and basophilic punctates may also be seen more or less in the early stages of anemia, but they are rather inconstant or few in number. These results, as is well known, indicate an abnormal activity on the part of the bone-marrow in the sense of compensation for a loss of blood cells by hemorrhage.

Transfusion experiment. From the above recorded fact it is easily expected that if transfused red blood corpuscles may function in the

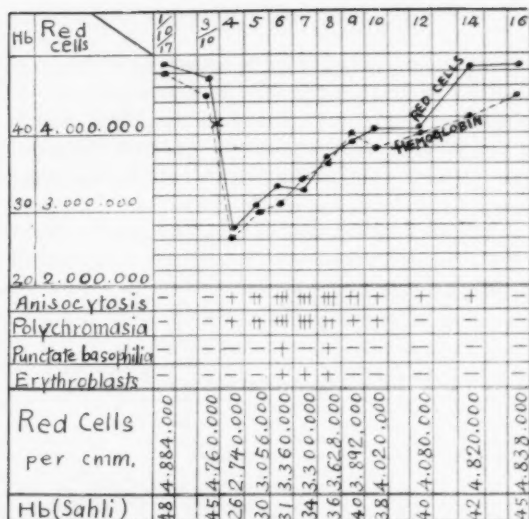


Fig. 1

* = bleeding; o = transfusion.

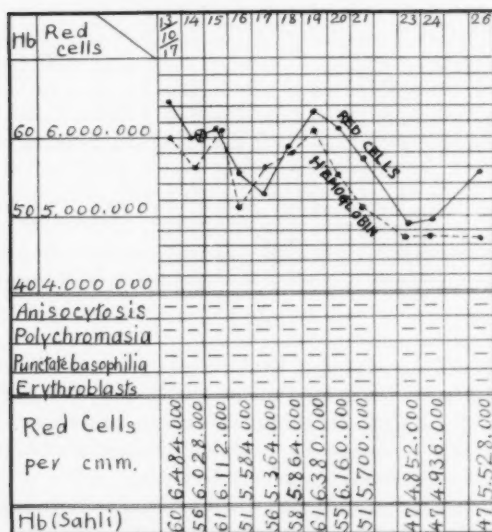


Fig. 2

animal body and an amount of blood is enough to make up for the previous deficit and if replacement is done as soon as the animal has been bled, there will not occur any change in the blood picture, neither numerically nor morphologically, the bone-marrow being subjected to no anemic stimulation, while in the reverse case such changes will take place.

In general we have noted the volume, the erythrocyte count and the hemoglobin of the drawn blood, and have calculated from these

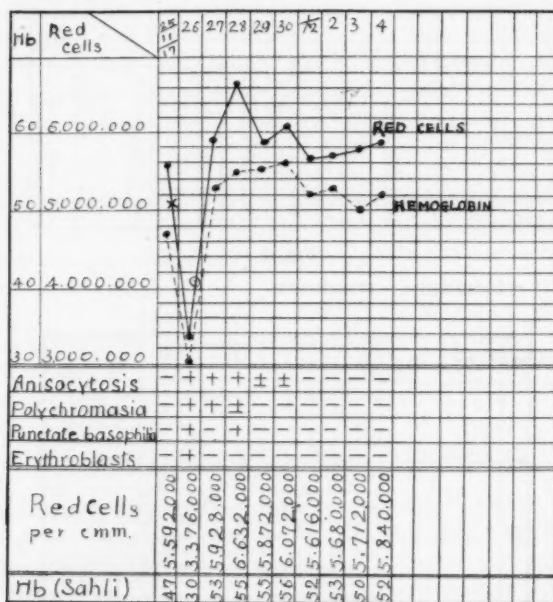


Fig. 3

the amount of cell suspension of known hemoglobin content that should be injected to restore the animal's hemoglobin content to the normal.

Experiment 2. Rabbit weighing 2000 grams was bled (40 cc.) from the carotid artery, and then 50 cc. of defibrinated fresh blood were injected at once. The animal was very lively.

In the case in which transfusion was done immediately after bleeding, neither hemoglobin content nor number of red cells indicates any change,

as figure 2 shows; morphological figures are entirely the same with the normal.

Experiment 3. The used rabbit weighed 3000 grams. Seventy-six cubic centimeters of blood were taken and after about 20 hours the same amount of defibrinated fresh blood was injected.

In the case in which blood has been replaced within twelve or twenty-four hours after bleeding, the hemoglobin content as well as the red cell count returned to normal level simultaneously with the injection, while the disappearance of polychromatophilia and anisocytosis took place after a few days, but in this case the degree of morphological change was milder and the duration of its appearance shorter than those of the control experiment (fig. 3). This fact illustrates that the bone-marrow was subjected to a transitory stimulation, a certain time having elapsed before injection.

As is evident from the results of the above experiments, the transfused red blood corpuscles are capable of functioning.

Among these latter experiments we met with two very interesting instances, that is, the animal dealt with as just described developed a sudden anemia on the fourth day after the blood pictures had once returned to normal. Furthermore polychromatophilia and anisocytosis in a large number, corresponding to the number of red cells, appeared again in circulation. This anemia returned to normal condition in the course of fourteen days as in the case of the experiment 1 (fig. 4).

Experiment 4. Rabbit of body weight of 2750 grams was bled 60 cc. at one time from the carotid artery. After four hours 65 cc. of defibrinated blood kept in ice for forty-eight hours were introduced. Animal remained in very excellent condition, very lively.

These results indicate evidently that the transfused red cells were destroyed on the fourth day after transfusion. We shall refer to the possible reason for this later.

Our study will turn now to determine how long these red cells are kept alive from the view of their capacity of functioning in the animal body. Landois, who has studied this problem extensively, states that cells kept for two days at 5°C. exert no harmful effect on the animal, but those kept for three days give rise to albuminuria and hematuria and cause death on the second day after transfusion. In 1916 Rous and Turner (4) developed a method for preserving living red blood cells in vitro and determined the length of their life by means of transfusion (5). This is the only report which paid attention to the morphology, so far as we are able to find in the literature.

They found that erythrocytes preserved in mixture of blood, sodium citrate and water for fourteen days remained in circulation and functioned so well that the animal showed no disturbance, and the blood count, hemoglobin and percentage of reticulated cells remained unvaried, while cells kept for twenty-three days, though apparently intact and unchanged when transfused, soon left the circulation.

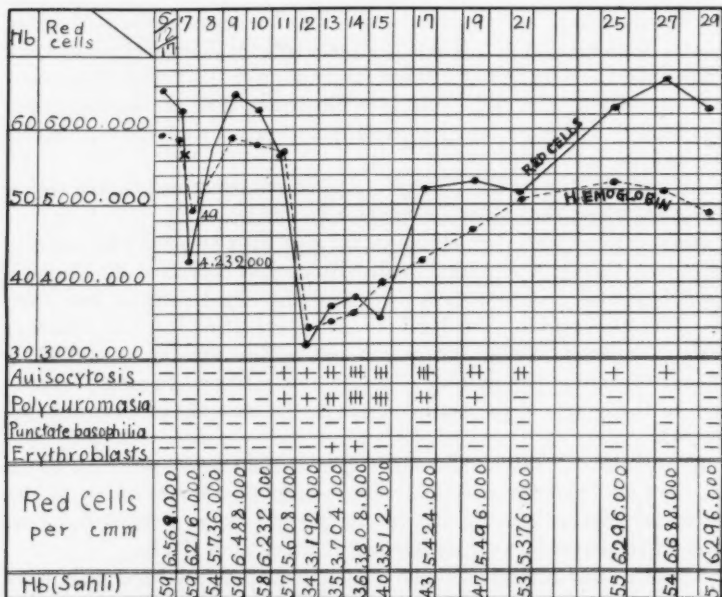


Fig. 4

Our experiments were made with defibrinated blood and blood-citrate-dextrose mixture to determine the availability for functional use of red cells kept in vitro.

Experiment with defibrinated blood. When sterile blood was put at once on ice it showed no trace of hemolysis in the supernatant fluid after ten days, while on the twentieth day of preservation a very slight hemolysis was seen, but the number of red cells did not show a marked

decrease, and the morphological character of the cells seemed entirely normal. When blood was kept for thirty days, there was some hemolysis and also an appreciable decrease in number of red cells. In the fresh and stained preparations the blood appeared normal. In experiments 5, 6, 7 and 8 the blood thus preserved for various periods was transfused.

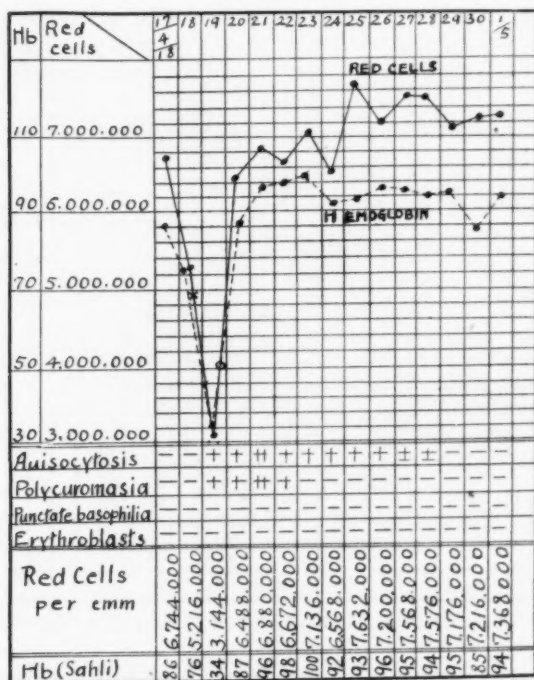


Fig. 5

Experiment 5. Rabbit weighing 2300 grams was bled twice (55 and 35 cc.). After twenty-four hours the equivalent amount (hemoglobin content 90 per cent) of defibrinated blood kept for three days was injected.

Experiment 6. Rabbit weighing 2050 grams was bled (40 cc.) and twenty-four hours later 38 cc. of blood which had been taken eleven

days previously were injected (number of cells in transfusate 5,456,000 in c.mm.).

Experiment 7. Body weight: 2100 grams. The recipient rabbit was bled (50 cc.) and at once the same amount of blood preserved for twenty days was transfused. The animal manifested at no time symptoms of distress.

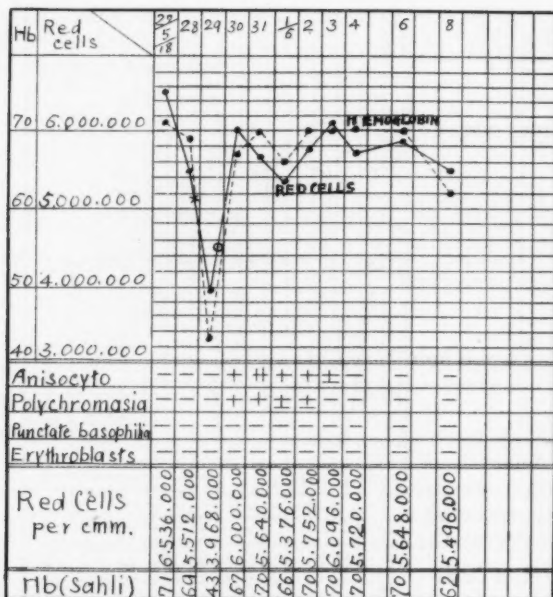


FIG. 6

As shown in charts 5, 6 and 7, red blood cells in defibrinated blood are capable of functioning even when they have been kept in vitro for three weeks.

Experiment 8. Experiments were all done with cells kept for thirty days in vitro.

A. An animal weighing 3150 grams was bled twice (70 cc. and 30 cc.), and an equivalent amount of kept cells was injected. The rabbit lived for two days but neither the number of red cells nor the hemoglobin content returned to normal. In morphological figures there

appeared noteworthy alterations corresponding to this change (fig. 8, A).

B. Body weight 1900 grams. The animal was bled 38 cc. and then 50 cc. of blood (cell count in c.mm.: 3,112,000) were introduced. This rabbit was alive for a long time after the operation, without showing any increase in number of red cells and hemoglobin percentage in consequence of transfusion. Thus in natural recovery of above mentioned anemia, they returned to normal in the course of ten days. At this time an appreciable change in morphology was noted.

Two other animals died within one-half or one hour after transfusion.

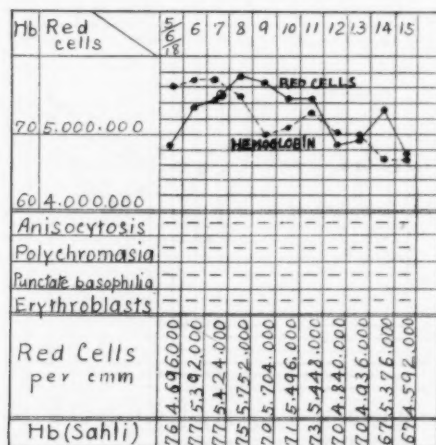


Fig. 7.

Both animals contained pitch-dark colored hemoglobinuria in the bladders.

Transfusion with cells kept in a blood-citrate-dextrose mixture. For the purpose of preservation three parts of the blood were mixed with five parts of isotonic citrate and two parts of isotonic dextrose solution. When they were to be used the supernatant fluid was pipetted off, the cells suspended in 0.85 per cent sodium chlorid solution until the original quantity of blood was reached and then used for injection. The mixture could be kept for one month without showing any trace of hemolysis. On the thirtieth day there began to appear a very slight hemolysis.

Experiment 9. Rabbit weighing 2500 grams was bled (50 cc.) and replaced after twenty-four hours with 60 cc. blood (number of blood corpuscles 4,516,000 in c.mm.) which had been kept for thirty days.

As figure 9 illustrates, the result was entirely similar to those cases in which fresh blood was transfused.

Experiment 10. A rabbit weighing 2850 grams was used. Two bleedings (60 cc. and 35 cc.) were effected; after having left the animal

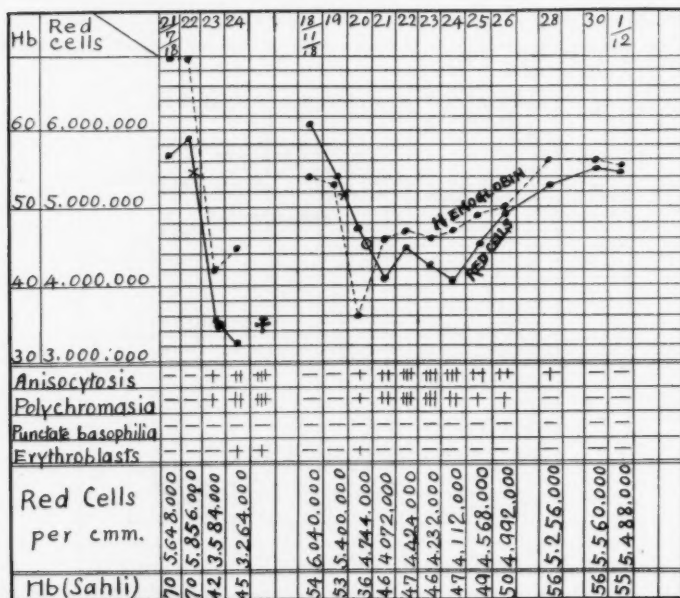


Fig. 8

in anemic condition for forty-eight hours, there appeared a large number of young cell forms in the circulation. After transfusion with an equivalent amount of blood cells preserved in ice for forty days, these cells disappeared within a few days from the circulation, indicating that the transfused blood corpuscles function entirely normally (fig. 10).

In this experiment it happened that the red cell count showed a gradual decrease from the fifth day after transfusion, reaching its maximum drop on the ninth day, after which a gradual recovery ensued. Mor-

phological figures ran parallel to the changes in number of red blood corpuscles. Marked polychromatophilia and anisocytosis, etc., which once had been restored to normal, appeared again on the tenth day, but at the time of recovery from anemia had disappeared altogether.

The experiments so far have shown that red cells may preserve their vitality in vitro for a long period, namely, in defibrinated blood for twenty days, in the mixture of blood-citrate-dextrose even for thirty days or more, and may function normally in the animal body when transfused. Our results therefore differ somewhat from the findings of

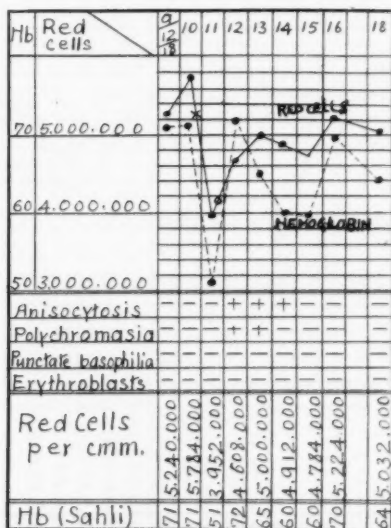


Fig. 9

Rous and Turner who concluded that cells kept for twenty-three days in their preservative mixture, though apparently intact and unchanged, soon left the circulation.

Preceding the conclusion of this work we will touch briefly on the cause of the peculiar cases of acute anemia above mentioned (exper. 4), which have developed at a certain period after transfusion. At first we assumed that this anemia may occur due to the destruction of red cells, being caused by the injurious effect on the function by cold, because

such transfusion experiments with blood preserved in ice showed in succession the same results, having initiated a sudden drop of hemoglobin on the fourth day after transfusion. But it became evident afterwards, as a number of experiments proved, that this destruction of red cells is not simply due to the preservation or the refrigeration by ice. Rous and Robertson (6) made a report similar to ours, having injected 10 cc. of blood every other day. They stated that in several animals

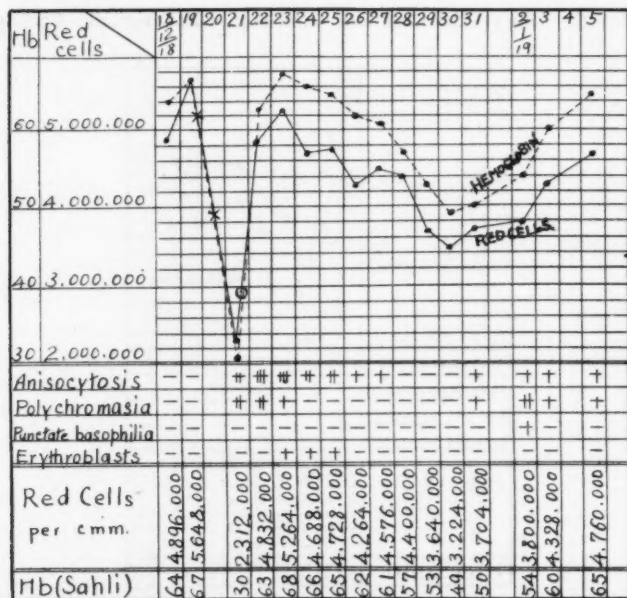


Fig. 10.

in which the agglutinin was strongest, the plethora was suddenly succeeded by severe anemia, despite continued transfusion.

Not having made any serological researches, we hesitate to assert whether or not in our cases such strong agglutinin was also existing. We could, however, demonstrate afterwards occasionally in a number of transfusion experiments such hemoagglutinin, though not so strong, similar to that pointed out by the above authors with regard to the character of temperature control of the agglutination, the persistence

of the agglutinating principle and many other points. Among those cases we had one instance (exper. 10) which developed anemia on the fifth day after transfusion, but more slowly than the acute cases above stated, reaching its maximum in the course of four days.

The plasma of this rabbit agglutinated not only the cells of its own, but also those of the same species and showed this activity even when diluted 150 times; it was also disposed to have stronger activity, in spite of a gradual recovery of anemia.

Rous and Robertson reported:

We have chilled, without result, two plethoric rabbits possessing a weak agglutinin in the hope of initiating a drop in the hemoglobin. The chilling was accomplished by means of ice-cold water, in which the well shaved ear of the rabbit was submerged for $\frac{1}{2}$ or 1 hour. Throughout this period the circulation in the cold ear was exceptionally good. The rectal temperature fell to 37°C., considerably below the normal for the rabbit, but not enough to produce the *in vitro* agglutination of blood corpuscles.

It seems probable that the agglutinin above described including also the case of Rous has no direct causal relation to such anemia and the question arises whether an uncommon hemolysin may play perhaps an important rôle.

Furthermore the fact that such occurrence has been noticed only in the experiments performed in cold winter time and not in other seasons, leads us to believe that it may have some connection with paroxysmal hemoglobinuria.

SUMMARY

1. Transfused red cells even preserved in ice for a long time, not to mention fresh blood, are capable of functioning if transfused into the animal body of same species.

2. Erythrocytes preserved as defibrinated blood maintain their normal vitality for twenty days, in the mixtures of isotonic sodium citrate and isotonic dextrose, even for thirty days and more; thus they may be used to replace the blood lost.

3. A sudden anemia occasionally develops a few days after transfusion. There is a possibility that this is due to the isolysin, even though it occurred only in isolated cases. We are, however, unable to draw a sure conclusion from the present work, but will pursue further our studies which may throw some light on this point.

The authors desire to express their thanks to Prof. Dr. T. Irisawa for his suggestions and encouragement throughout the course of this work; and also to Prof. S. Mita for his kind advice.

BIBLIOGRAPHY

- (1) PANUM: Virchows' Arch., 1863, xxvii, 240.
- (2) PONFICK: Virchow's Arch., 1873, lxii, 273.
- (3) LANDOIS: Transfusion des Blutes, Leipzig, 1875.
- (4) ROUS AND TURNER: Journ. Exper. Med., 1916, xxiii, 219.
- (5) ROUS AND TURNER: Ibid., 1916, xxiii, 239.
- (6) ROUS AND ROBERTSON: Ibid., 1918, xxviii, 509.

ON THE REGENERATION OF THE VAGUS NERVE

F. T. ROGERS

From the Hull Physiological Laboratory, University of Chicago

Received for publication April 30, 1920

Three years ago, in connection with work on nerve crossing between the phrenic and vagus, attention was again directed to the doubt existing as to the possibility of functional return after section and suture of the vagus. This, according to Langley (1) and Tuckett (2), and more recently by Schaffer (3) is doubtful or, if it occurs, it does so only after the lapse of years. As a control on other work it was decided to repeat the old experiment of section and suture of one vagus leaving the other intact and after as long a time for recovery as practicable in the laboratory, test the nerve for functional activity. In order to avoid the uncertainties involved in testing functional regeneration by electric stimulation, resort was made to the old suggestion of subsequently cutting the intact vagus leaving the regenerating nerve to exert such action as it might. This conclusion was forced by the negative results following stimulation of the nerve when only a few months were allowed for regeneration. The results of this series of experiments confirmed the similar experiments made by others using the same method (1), (4).

The first series of experiments consisted of four cats and one dog in each of which one vagus and cervical sympathetic were sectioned and sutured just below the level of the thyroid gland. After time intervals of from three weeks to six months the nerve was tested electrically with the animal under ether anesthesia and with arterial pressure recorded from the carotid artery (table 1). In every case it was found that while stimulation of the normal nerve produced the usual cardiac inhibition and fall in blood pressure, the stimulation of the previously sectioned and sutured nerve caused no cardiac inhibition and no gastric motor effects when stimulated on either side of the point of suture. Stimulation of this trunk above and below the scar did cause the usual respiratory inhibition and gave reflex effects on the blood pressure. With reference to the actual recovery of efferent fibers, Tuckett states

that after three years this does occur. The only other similar positive statement that I have found is that of a single observation of Stewart (5), of which he states that "some regeneration appeared to have taken place (after three hundred days) since stimulation of the nerve caused slowing and weakening of the heart."

TABLE 1

In each animal of this series one vagus was sectioned and sutured, except in the case of cat 17 in which the nerve was crushed by hemostatic forceps and not sectioned. After the time intervals indicated in the table, the animals were etherized, carotid blood pressure tracings made and both the normal and the previously sectioned vagi were stimulated with tetanizing current. Stimulation was applied above and below the scar of union, but in no case did the effects differ. In all the animals of this series it was found at autopsy that the two ends of the nerve were united by a small neuroma

ANIMAL	TIME ALLOWED FOR REGENERATION	EFFECTS OF STIMULATING THE SUTURED VAGUS			
		Heart	Respiration	Blood pressure	Stomach
	<i>months</i>				
Cat 28	2	No inhibition	Inhibition		No contractions
Cat 12	4	No inhibition	Inhibition		
Cat 18	5	No inhibition	Inhibition	Weak stimulation pressor effect, strong stimulation depressor effect	
Cat 17	6	No inhibition	Inhibition	Depressor effect	No contractions
Dog 40	4	No inhibition	Inhibition		

Of the second set of animals only two dogs survived the long time set for the experiment (tables 2 and 3.) In two dogs, nos. 95 and 96, twenty and sixteen months respectively elapsed between the section of the one nerve and the section of the remaining vagus. In one case the animal lived thirty-four days and in the other sixteen days after cutting the second nerve. The cause of death in both cases seemed to be starvation due to paralysis of the esophagus and continued vomiting which followed attempts to eat. Respiration continued at a normal rate and amplitude during this interval of life, save that at times both dogs showed a hiccough-like disorder associated with cough that seemed to be due to irritation of the respiratory tract by vomited material. This continuation of normal breathing might be considered

confirmatory of Schaffer's recent findings that section of the vagi in the cat causes little change in the respiratory rhythm provided asphyxia be prevented by keeping the larynx open. The fact noted in the tabulated findings that in one of my animals the section of the regenerated nerve after the previous section of the other vagus was followed by a slowing and deepening of the breathing, indicates regeneration had occurred, of either or both, the afferent pulmonary fibers or motor fibers to the laryngeal muscles. According to Vanlair (6), functional regeneration of the motor fibers of the recurrent laryngeal nerve can be demonstrated after one year. The facts just stated above seem to confirm this finding of Vanlair but unfortunately the writer was unable in this dog to make any observation as to the part played by the larynx in this change of respiration.

With reference to the heart an interesting condition was found. Electrical stimulation of the regenerating vagus, with the animal under ether anesthesia, caused no cardiac inhibition. Sectioning of the normal nerve so as to leave only the regenerating nerve in relation to the heart, was followed by a marked increase in the rate of the heart beat. These facts of negative results to electric stimulation and an immediate increase of the heart rate after cutting the remaining normal nerve indicate that the regenerating nerve was not functional for it is common knowledge that section of only one vagus leads to only a slight cardiac acceleration. This conclusion was subsequently confirmed by cutting the regenerating nerve which caused no change in the heart rate (table 2).

In spite of these indications of absence of function in the regenerating fibers, the rate of the heart beat daily became less and in two weeks the rate was that of a normal animal. In other words, with one vagus degenerated and the other not functional, the cardiac rhythm returned to a normal rate. This fact was also noted by Stewart.

When this stage of recovery had been reached, the injection of atropine gave a tremendous increase in the heart rate. This effect was evidently due to some other factor than that of paralysis of vagus fibers. The writer hesitates to speculate on the mechanism of this atropine effect. It recalls the observation of Carlson (7) that atropine stimulates the heart ganglion of *Limulus* and suggests that the usual effect of atropine in the normal animal is twofold, paralyzing the extrinsic inhibitors and stimulating the automatic nerve mechanism.

In dog 95 a fortunate incident gave direct ocular proof of the fact that the vagus inhibitory fibers to the heart can regenerate. As stated

TABLE 2

Dog 96

July 4, 1918. Section and suture of the right vago-sympathetic. The dog is three and a half months of age
 October 15, 1919. Gastric fistula is made

DATE	HOOR	PROCEEDING	RESPIRATION	HEART RATE	REMARKS
Nov. 1	2:00 p.m.	Dog quiet	12 per min.	84	
Nov. 1	5:00 p.m.	Left vagus sectioned			
Nov. 1	10:00 p.m.		12 per min.	230	Vomiting during the night
Nov. 2	11:00 a.m.		20 per min.	162	
Nov. 2	9:00 p.m.		9-12 per min. irregular		
Nov. 3			14 per min. irregular	130	Eating without vomiting
Nov. 4			13 per min. irregular	142	Eating and vomiting
Nov. 5			8 per min. labored	142	Vomiting and cough
Nov. 6			9 easier	146	Alert, wags tail. Vomits and coughs
Nov. 7			10 per min.	136	Eats nothing
Nov. 11	3:00 p.m.	Stomach traacing made	10 per min.	116	Getting very thin
Nov. 11	4:30 p.m.	0.3 cc. of 0.1 per cent atropine			
Nov. 11	5:00 p.m.			149	
Nov. 12	1:00 p.m.		10 per min.	114	Very thin, eats nothing
Nov. 12	5:00 p.m.	Cut the right vagus			
Nov. 12	11:00 p.m.		5 per min.	112	
Nov. 14	2:00 p.m.		6 per min.	92	
Nov. 14	3:00 p.m.	0.3 cc. atropine 0.1 per cent			
Nov. 14	3:15 p.m.		6 per min.	160	
Nov. 14	6:00 p.m.		6 per min.	122	

Nov. 16, 2:00 p.m. Dog dead. Lungs, hyperemic patches with two pus pockets, $\frac{1}{4}$ inch diam. Scraps of food in stomach. The two ends of the vagi united.

Observations at time of cutting the second vagus, November 1, 1920.

Blood pressure recorded from the left carotid artery.

Balloon in stomach connected with water manometer, to record gastric contractions and respiration.

Dog under ether anesthesia: stimulation with tetanizing current.

1. Effects of stimulating left vagus before the nerve was cut.

Heart, inhibition and fall of carotid pressure (fig. 1, A). Respiration, inhibition.

Stomach, strong contraction followed by weaker ones (fig. 1, A).

2. Effects of stimulating the right nerve which had been cut and sutured. Stimulation central to the scar of union.

Heart, no inhibition. Rise in blood pressure.

Stomach, no contraction.

Respiration, inhibited.

Stimulation peripheral to scar of union.

Heart, no change. Rise in blood pressure (fig. 1, B).

Stomach, no contraction (fig. 1, B).

Respiration, inhibited.

3. Repeat the stimulation of the normal nerve.

Normal effects on heart and stomach, as in paragraph 1 above.

4. Cut the left vagus and closed the wound in the neck.

previously, the dogs seemed to die of starvation. Two or three days before death the animals became inactive and went into a comatose condition. In the case of this animal, I chanced to find him when death was imminent. The dog was cold to touch, breathing was barely perceptible at three or four times per minute. Since the animal was dying, it was killed by opening the thorax without anesthesia. The heart was beating slowly at the rate of 40 per minute. The regenerating vagus trunk was stimulated by tetanizing current, twice above and twice below the scar marking the point of suture. Each time of stimulation the heart ceased beating for five to ten seconds and resumed beating at a slower rate than that preceding the stimulation. Unless there be accessory cardiac fibers outside the main vago-sympathetic trunk, which had escaped section, this observation indicates that regeneration of the vago-inhibitory fibers may occur if sufficient time be allowed. And it lends force to the criticism that electric stimulation of regenerating nerves in anesthetized animals is not a wholly reliable test.

In dog 96 a gastric fistula was made a month before cutting the second vagus. When this animal was etherized at the time of cutting the second nerve, comparative graphic tracings were made of the motility changes in the stomach after stimulating the normal and the regenerating nerve. Under light anesthesia, stimulation of the normal

vagus caused a strong contraction followed by smaller peristaltic waves. Stimulation of the regenerating vagus a few minutes later caused no detectable contraction of the stomach (fig. 1). Two weeks

TABLE 3

Dog 95. Adult brown bull dog

March 7, 1918. Sectioned the right vago-sympathetic and dropped the ends together in the carotid sheath. The sheath was then closed by two stitches, but none were taken through the nerve ends
November 5, 1919. The left vago-sympathetic was sectioned

DATE	HOUR	PROCEEDING	RESPIRATION	PULSE	REMARKS
Nov. 5	1:00 p.m.	Before operating	15	74	Dog active
Nov. 5	2:00 p.m.	Cut left vago-sympathetic			
Nov. 6			14	147	Eating. Chases guinea pig
Nov. 7			12	142	Drinks water; vomits solid food
Nov. 15			12	124	Eating and vomiting
Nov. 17		Dog emaciated	15	146	Drinks milk
Nov. 18			12	112	Frequent hiccough
Nov. 19	2:00 p.m.		12	108	Dog shivering, hiccough
Nov. 19	2:45 p.m.	Given 0.3 cc. of atropine sulph.			
Nov. 19	3:15 p.m.		12	186	
Nov. 19	7:30 p.m.		12	120	
Nov. 20			16	129	Dog eats and then vomits. Is very thin
Nov. 21		Dog is fed soft food only			Vomiting reduced
Dec. 1					Continued progressive emaciation
Dec. 8		Will not eat			Lies quietly; does not move about
Dec. 9		Thorax opened, vagi stimulated			Dog comatose
Dec. 9		Autopsy			

Lungs have scattered hyperemic areas but no consolidation. One small pus pocket found. Stomach empty, normal appearance. Heart normal. The two ends of left vagus united by a smaller strand of tissue.

after cutting the second vagus, tracings were made of the hunger contractions in this dog (fig. 2). These were similar to those occurring before cutting the second nerve but not so vigorous. These contrac-

tions were promptly abolished by atropine. This is suggestive of direct paralysis of the vagus fibers in the regenerating nerve but it does not prove that regeneration of the gastro-motor fibers had occurred, for this inhibition might have been due to any of the following possible factors: *a*, a direct action on the intrinsic plexuses as suggested by Magnus for the intestine; *b*, inhibition through the splanchnics as suggested by Magnus for the intestine; *c*, or some possible rela-

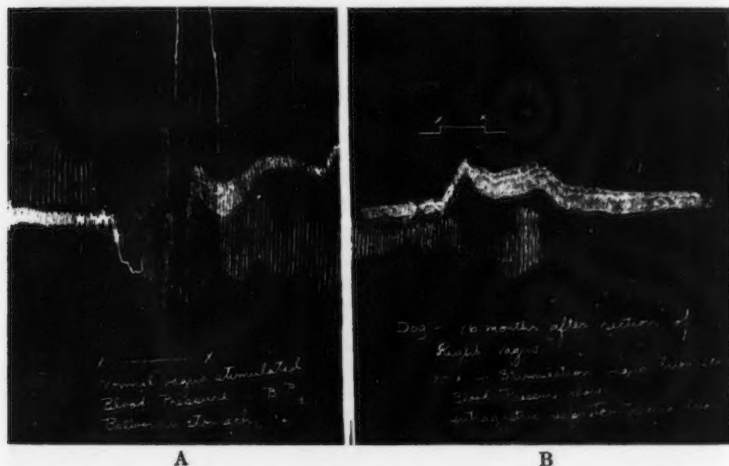


Fig. 1. A. Stimulation of normal vagus, dog 96. Carotid blood pressure and balloon in stomach to record respiration and stomach contractions. The stomach tracing is above the blood pressure record on the left side of figure A and drops below on the right side. Cardiac inhibition, respiratory inhibition and contraction of the stomach followed stimulation of the nerve.

B. Stimulation of the right vagus sixteen months after section and approximation of the ends of the nerve. No cardiac inhibition, no stomach contraction, inhibition of respiration and a rise in blood pressure followed stimulation of the nerve below the scar of union. Ether used as anesthetic.

tion to the secretion of epinephrin. All other evidence in this dog indicated that these gastric fibers were not functional and hence the observation indicates that atropine will inhibit gastric contractions independently of whether or not the vagus fibers are active. At any rate, electric stimulation of the regenerating nerve in the anesthetized animal gave no gastric motor effects and atropine abolished gastric motility in the unanesthetized condition.

In these dogs in which a year and a half was allowed for the regeneration of one vagus after section and suture, the subsequent division of the remaining nerve was followed by no appreciable change in the rate of breathing or in the amplitude of the respiratory movements, so far as could be judged by ocular observation. Stimulation above and below the scar of suture with a tetanizing current caused the usual

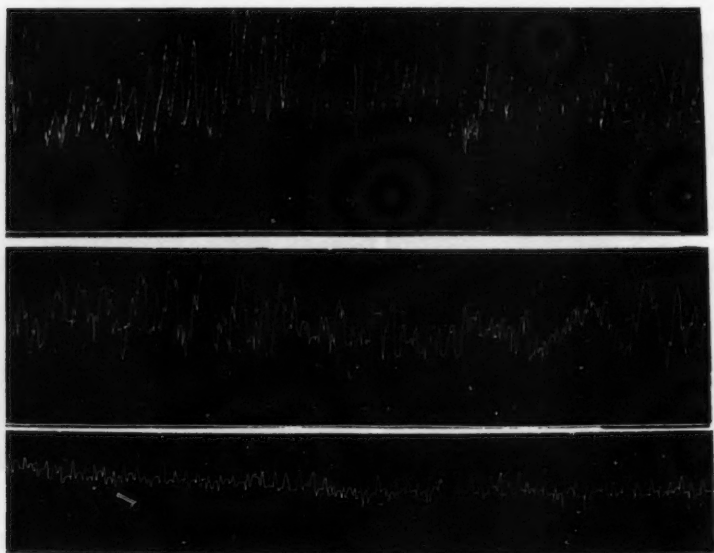


Fig. 2. Tracings numbered I, II, and III in order from above, downwards. I. Gastric hunger contractions, dog 96, October 28. Right vagus sectioned and sutured sixteen months previously. Left vagus intact.

II. November 11, gastric hunger contractions after cutting left vagus leaving only the regenerating nerve intact.

III. November 11. Continuation of tracing II. Thirty minutes after a subcutaneous injection of 0.3 cc. of 0.1 per cent atropine sulphate.

nhibition of breathing. Although causing no cardiac inhibition when electrically stimulated in the etherized dog there was an immediate pressor effect on the blood pressure (fig. 1). Regeneration of afferent fibers in the vagus had therefore occurred.

After death the regenerated nerve of dog 95 was excised for a distance of half an inch above and below the point of suture. This was

stained by Ranson's pyridine silver method for medullated and non-medullated fibers. Save that the arrangement of nerve fibers in fascicles below the scar was not evident there was no distinct difference in the number of nerve fibers in the regenerated part as compared with that above the point of section.

In this report no reference is made to the changes in the sympathetic nerves of the neck which were cut simultaneously with the vagi.

SUMMARY

One vagus nerve was sectioned and the ends approximated so as to allow regeneration to occur in a series of dogs and cats. The regenerating fibers were stimulated electrically at time intervals varying from one to sixteen months after cutting. These tests made with the animals under ether anesthesia gave no evidence of the regeneration of either cardiac inhibitory or gastric motor fibers.

In one dog twenty months after one vagus was sectioned, this nerve was stimulated with the dog in a comatose condition but no ether anesthesia. Distinct cardiac inhibition followed.

In two dogs, section of the remaining normal vagus, sixteen and twenty months after previously sectioning and suturing the other, led to death in sixteen and thirty-four days respectively. Apparently death was due to starvation resulting from difficulty in swallowing and frequent vomiting. During the period of life following section of the second vagus, the following facts were noted:

1. An immediate marked increase in pulse rate followed section of the second vagus. This slowly declined and after eleven to fourteen days the rate was that of a normal animal. At this stage atropine caused a great increase in the rate of the heart beat. These effects occurred in a dog in which the regenerating nerve was not functional for subsequent division of the nerve caused no change in the heart rate.

2. With only the regenerating nerve intact, but with no evidence of it being functional, atropine reduced the gastric motility.

3. The rate of breathing with only the regenerating nerve intact was the same as it was with one vagus intact. Cutting the regenerating nerve led to the classic picture of slow labored breathing. Stimulation of the regenerating nerve above and below the scar caused the normal respiratory inhibition and pressor effects on the blood pressure. Regeneration of the vagus fibers necessary to maintain the normal

respiratory rhythm had therefore occurred. Whether these were motor to the larynx or afferent from the lungs was not determined.

After bilateral vagotomy, some compensatory process is set up whereby the pulse rate is brought back to normal in spite of the absence of the vagi.

BIBLIOGRAPHY

- (1) LANGLEY: Journ. Physiol., 1895, xviii, 283.
- (2) TUCKETT: Journ. Physiol., 1900, xxv, 303.
- (3) SCHAFFER: Quart. Journ. Physiol., 1919, xii, 231.
- (4) PETIOSKY AND TSCHERMAK: Arch. gesamt. Physiol., 1913, clii, 523.
- (5) STEWART: This Journal, 1907, xx, 407.
- (6) VANLAIR: Arch. d. Physiol., 1894, vi, 217.
- (7) CARLSON: This Journal, 1906, xvii, 177.

EFFECT OF VARIOUS SUBSTANCES UPON THE COAGULATION OF CITRATED PLASMA¹

BEN KARPMAN

From the Department of Pharmacology, University of Minnesota

Received for publication May 3, 1920

Although an enormous literature has arisen upon the coagulation of the blood, and the rôle of inorganic salts, lipoids and tissue extracts has been extensively investigated, little has been done along the line of the organic substances. I have therefore undertaken, in this paper, a systematic investigation of the effects of as many classes of organic compounds as possible upon the coagulation of citrated blood plasma. It was hoped to determine by this method whether any substances or radicals could be found which might be regarded as specifically favoring or specifically inhibiting the act of coagulation, and which might thus throw light upon the intimate chemical mechanism of coagulation.

METHOD

Fresh beef blood taken from the abattoir at the time of slaughter was put into a jar containing enough sodium citrate solution to make the final mixture contain 0.5 per cent of the salt. This was centrifuged, put in the ice box and used the same day.² A 1 per cent solution of

¹ Thesis submitted for the degree of M.D., University of Minnesota.

² Effect of age on the activity of citrated plasma. I. *Citrated plasma* was tested for coagulation time,—when fresh, and also when 1, 2, and 3 days old. Coagulation time was found to be 5 minutes, 35 seconds; 8 minutes; and 7 minutes, 15 seconds, respectively. The precipitate formed during these intervals was not removed.

II. Citrated plasma was tested for coagulation time when fresh, 11 days, 14 days, 16 days and 21 days old. Precipitate formed removed in the intervals mentioned. Coagulation time was 5 minutes, 35 seconds, and 4 hours for the first two; the rest formed within a day a small amount of solid material which was suspended in the fluid.

III. Citrated plasma was divided into three portions and tested for coagulation time in the intervals indicated. Precipitate removed each time. Normal clotting time, 13 minutes.

a. Tested when 2, 6, 7, 8 and 9 days old. Coagulation time 15 minutes, 55 sec-

dry crystals of CaCl_2 in distilled water was made, and this was added to the plasma at the same time as the substance whose effect on coagulation was being studied.

The tubes used were mostly small Wassermann tubes. Where larger tubes were used they were, whenever possible, selected of the same size to insure uniformity of contact with foreign material. Observations were all made at room temperature, usually 72°F . It is to be noted, however, that during the winter and spring months the average coagulation time of controls was 8.1 minutes (the highest being 13.4 minutes and the lowest being 3.6 minutes), while during the summer months the average coagulation time of the plasma was 5.6 minutes

onds; 19 minutes, 15 seconds; 21 minutes, 36 seconds; 3 hours and 6 hours respectively.

b. Tested when 6 and 8 days old. The first gave a coagulation time of 40 minutes, 15 seconds; the second was still fluid after 12 hours.

c. Tested when 8 days old. Still fluid after 24 hours.

IV. Citrated plasma was tested for coagulation time when fresh, 4 days, 5 days, 9 days and 10 days old. At the intervals indicated, the citrated plasma was divided into two portions; a larger one containing the clear supernatant fluid and a smaller one containing all of the precipitate formed during the interval. The former was treated again in the same manner on successive days as more precipitate formed. Normal coagulation time 4 minutes, 10 seconds. The larger (supernatant) fraction gave a coagulation time of 3 minutes, 24 seconds; 8 minutes, 28 seconds, and 8 minutes, 42 seconds, on the 4th, 9th and 10th days respectively. The first small (precipitate-containing) portion gave a coagulation time of 2 minutes, 37 seconds on the 4th day, and spontaneous clotting on the 5th day; the second small portion, 3 minutes, 47 seconds on the 9th day; the third small portion, 5 minutes, 52 seconds on the 10th day.

V. An extract was prepared from fresh citrated plasma by a method modified from Wright (1). The plasma to which this extract was added showed some reduction in coagulation time. The results were vitiated by the fact that the extract was used in a solution of sodium carbonate; as the latter substance interferes with coagulation, it masked the effect of the extract.

The above observations suggest that the coagulation time of citrated plasma increases considerably with age. This increase is more marked when the precipitate formed in the intervals is removed, but when the precipitate is present in larger amounts, the coagulation time is markedly shorter. This fact suggests that the precipitate contains something which aids coagulation; but whether this is due to the presence in the precipitate of a definite thromboplastic substance, or is merely due to hydrolytic dissociation of calcium citrate with liberation of calcium ions, or to some other entirely different factor, has not been determined. A substance extracted from citrated plasma also showed coagulating power; but it is not clear yet whether this substance is identical with other kephalin like substances that can similarly be extracted from various organs and tissues.

(the highest being 8 minutes and lowest being 2.3 minutes). Whether such marked variation is due to heat or to some nutritional factor that in some way affects coagulation time in winter as compared with summer, has not been determined. To insure uniformity and greater accuracy, the plasma, CaCl_2 solution and the substance used (when liquid) were all measured from microburettes graduated to 0.1 cc. The plasma was measured out first and then the Ca solution was added. Immediately afterward the chemical to be tested was added, and the tube moderately shaken and mixed. The end point was judged by Howell's method inverting the tube, and time noted in minutes and seconds. Occasionally we experienced a little difficulty with this criterion, since some of the substances would give only partial or incomplete coagulation.

A new control tube was used for each set of experiments and for each sample of plasma.

Throughout the experiments care was taken to test the effects of single substances rather than of mixtures, but this was not always possible, a fact which must be borne in mind in the interpretation of results. The effect of water on coagulation time of plasma has been determined in a series of experiments, and it was found that there is a definite, although not very marked, diminution in coagulation time. It is clear, however, that these results cannot be applied entirely to solutions of substances, since these in solutions behave somewhat differently than when pure, even aside from the mere factor of water. Where mixed salts were used, such as choline HCl or tyramine HCl, the results cannot be applied entirely to choline or tyramine without reservation, since the acid factor might mask their effect.

In the descriptions which follow, the word "clot" is used to designate the formation of a single coherent jelly-like mass which adheres to the walls of the tube; the word "precipitate" is used to designate the formation of discrete flocculi which do not cohere to one another and do not form a jelly. This use of these terms must be borne in mind in the interpretation of the results recorded in this paper.

RESULTS OF EXPERIMENTS

Group I. Aliphatic series

1. *Formic acid.* All tubes formed thick viscous fluids. No distinct precipitate could be seen. The time of solidification (coagulation) was in direct proportion to concentration. The solid resembled more a precipitate than a clot and appeared homogeneous and structureless.

2. *Acetic acid.* All tubes gave a very flocculent precipitate, the amount of the latter being in direct proportion to concentration. With the exception of the 0.8 per cent which remained permanently fluid, the supernatant fluid of the rest of the tubes solidified after a few days; it was of a soft mushy consistency and no fluid could be expressed from it.

3. *Propionic acid.* All tubes became cloudy with the formation of a precipitate. The 0.85 per cent tube was still fluid after 2 hours; the rest of the tubes solidified in 2 minutes, 10 seconds; 15 seconds and 5 seconds, (control 5 minutes, 45 seconds), the substance being very soft and mushy.

4. *Butyric acid.* The 0.85 per cent became cloudy and emulsion-like with a fine flocculent precipitate, and remained permanently fluid. The 2 per cent gave a very soft solid after 2 days; the rest of the tubes formed almost instantaneously thick solid precipitates.

5. *Valeric acid.* The 0.4 per cent mixed well but remained permanently fluid with formation of a precipitate and cloudy supernatant fluid. The rest of the tubes did not mix well, forming thick emulsion-like fluids above and a large precipitate on the bottom. Of these, the 0.85 per cent was still fluid after 3 days; the 2 per cent was practically solid in 15 minutes; and the 4 per cent and 7.5 per cent formed almost instantaneously thick solid precipitates.

6. *Oleic acid.* On mixing an emulsion formed in the tubes. The contents of all tubes were still fluid after 24 hours with an evident tendency to form solid emulsions.

7. *Formaldehyde.* The clots were very soft, practically semi-solid³ and very transparent, the transparency being more marked in higher concentrations. No precipitate was visible and no fluid could be expressed. The solid was easily broken up into small particles.

8. *Alcohol.* All tubes became cloudy and milky in proportion to concentration, and a white precipitate separated out afterwards. The 7.5 per cent and 14 per cent tubes solidified in time equal to that of control. The other tubes remained permanently fluid. In firmness the clots resembled the normal.

9. *Glycerine.* In concentration of 0.85 per cent to 3 per cent showed some retardation, this being more marked in the lower percentages; in concentration between 4 per cent and 7.5 per cent no marked deviation from control was evident, while in concentration above 7.5 per

³ See footnote 4.

cent it again showed retardation, the effect increasing with increased concentration. The clots were very firm and elastic, and no serum could be expressed.⁴

10. *Ether*. The 7.5 per cent and the 14 per cent mixtures showed considerable retardation, the clots resembling normal in firmness and consistency. The 20 per cent and 30 per cent coagulated but partly in 30 minutes, the rest remaining fluid. As the substance was added it did not mix with the plasma and was seen gradually to rise to the top. In all cases, coagulation proceeded from the bottom.

11. *Acetone*. The 4 per cent, 7.5 per cent and 14 per cent tubes formed clear mixtures, which coagulated in 1 hour, 1.8 hour and 2.5 hours respectively. The 20 per cent, 25 per cent and 29 per cent formed turbid, emulsion-like mixtures with precipitation. The first two were found to be solid after 2 days, while the last one remained fluid. The clots were soft and mushy and no fluid could be expressed.

12. *Urea*. There was no precipitate visible. Clot softer than normal. Considerable retardation.

13. *Hexamethylenamine*. In all tubes, with the exception of 0.1 per cent, there was a settlement of the substance in proportion to concentration. There was no apparent difference whether it was used in solution or in pure form, for although the deviations in the latter were much more marked, coagulation time was practically normal. Clots were somewhat harder.

14. *Hexamethylenamine and phosphoric acid, 50 per cent of each*. All tubes were fluid at first with no evident signs of either coagulation or precipitation. In $2\frac{1}{2}$ hours became thick and semi-solid. Were all found to be solid in 15 hours, soft in consistency, with no fluid expressible, the solid resembling more a precipitate than a clot.

15. *Choline hydrochloride*. All tubes gave delayed coagulation time in proportion to concentration. The 5 per cent was still fluid after a half-hour.

16. *Glyocol*. In small concentrations up to 3 per cent the tubes became cloudy while in higher concentrations the tubes formed a distinct precipitate. After 18 hours the 0.3 per cent was solid, the 0.6 per cent semi-solid, the rest solid white precipitates.

⁴ In the course of the experiments it was frequently noted that there was a considerable difference in the effect of various substances on the character of the clot. Thus, glycerine gave a very firm and elastic clot; dinitrobenzol a soft clot, while resorcin gave a firm clot in concentration up to 0.5 per cent and a very soft one in higher percentages, and no fluid could be expressed.

17. *Chloroform*. Did not mix with the plasma and was seen floating in it as oily drops, gradually settling to the bottom. Coagulation proceeded from the top. The clots were soft, clear and jelly-like.

1. The first members of the saturated fatty acid series show considerable similarity in their effect on coagulation of plasma. They all interfered with coagulation by precipitation, the amount of the latter being in direct proportion to concentration; and the higher the member the more pronounced was the reaction.

2. Oleic acid interfered with coagulation by emulsification.

3. The representative members of the other group, namely, formaldehyde, alcohol, ether and acetone, have all produced the effect of retardation, interference, or both. The retardation produced by formaldehyde can hardly be explained by precipitation since it did not show any visible change; however, the character and consistency of the solid suggest that it was more like a precipitate than a clot.

4. Glycerine did not show any appreciable effect on coagulation, although its power of retarding coagulation in very low and very high concentration is suggestive.

Of the rest, glycerol markedly interfered with coagulation even in small concentrations. The effect of choline HCl and urea is considerable less marked while hexamethylenamine was practically without any effect. Chloroform has shown a definite retardation and possible inhibition.

Group II. Aromatic Series

1. *Benzol*. On mixing, all tubes assumed a milky emulsion-like appearance. The tubes solidified later, some showing a separation into three layers, benzol, emulsion and plasma, from the top down.

2. *Phenol*. In concentrations of 0.2 per cent to 0.85 per cent, normal or only partial coagulation was obtained, whether the phenol was used in pure form or in solution, although the process was more complete when the same strength was used in solution than in crystals. The clot formed was normal in appearance and spread from the top down. The 2 per cent to 8.5 per cent showed emulsification and precipitation, solidifying later with separation of fluid.

3. *Resorcin*. The 0.1 per cent to 0.4 per cent gave firm clots with delayed coagulation time; the 0.85 per cent gave a soft clot. The 2 per cent to 25 per cent assumed an emulsion-like appearance with subsequent separation of a precipitate, not unlike phenol, but the tubes remaining permanently fluid.

4. *Benzaldehyde*. On mixing all tubes became turbid and emulsion-like with formation of a precipitate, the thickness of the emulsion and the amount of the precipitate being in direct proportion to concentration. All tubes remained permanently fluid except the 0.85 per cent, which solidified very slowly.

5. *Benzoic acid*. The 0.1 per cent to 0.4 per cent and 2 per cent showed delayed or partial coagulation, in direct proportion to concentration, the clots being less elastic. The 0.85 per cent gave a precipitate and remained fluid.

6. *Benzyl alcohol*. All tubes showed emulsification and precipitation, in direct proportion to concentration.

7. *Nitrobenzol*. On mixing, all tubes became turbid, separating later a precipitate which settled to the bottom as a white solid mass, the amount of the precipitate being in direct proportion to concentration. The supernatant fluid was quite clear and coagulated, the coagulation time not varying markedly from normal.

8. *Dinitrobenzol*. In spite of shaking, a considerable part of the crystals settled to the bottom. Coagulation time was normal. No precipitate was visible.

9. *Cinnamic acid*. The 0.85 per cent gave normal coagulation time. The 2 per cent to 4 per cent formed a hard surface scum, the material below remaining fluid for a considerable time. Were all found to be clotted in 60 hours, the clots being paler in appearance and not as firm in consistency as normal.

10. *Aniline*. The 0.85 per cent gave delayed coagulation, the 2 per cent partial coagulation. The 2.4 per cent to 29 per cent formed emulsion-like mixtures.

11. *Phenylhydrazine*. On the addition of the substance there was an almost instantaneous precipitation and solidification, the solid being soft and mushy with no fluid expressible. The solid was very much unlike a clot and was easily broken up into small masses.

12. *Pyridine*. Tubes became cloudy, pale and soon solidified, the solid being easily broken up into small bits and masses, and resembling more a precipitate. No fluid could be expressed.

13. *Quinoline*. All tubes showed a precipitate on the addition of quinoline, the amount being in direct proportion to the amount of substance used.

14. *Tyramine hydrochloride*. Clots were apparently normal both as to color and consistency.

15. *Antipyrine*. All tubes formed a clear solution with no settlement of antipyrine or precipitate visible. The 4 per cent was still fluid

after a week, the 7.5 per cent was solid in 14 hours, the solid being very soft and flocculent. The rest solidified promptly on the addition of the substance, the solids being of soft gelatinous consistency and with no fluid expressible. The retardation was inversely proportional to concentration.

16. *Caffeine*. There was a settlement of caffeine on the bottom in proportion to concentration. Clots very clear, soft and no fluid could be expressed.

The benzol series showed a variable effect:

1. Benzol, aniline and benzaldehyde interfered with coagulation by emulsification. With phenol the effect is changed to that of precipitation, due to a change in solubility by the introduction of the OH group. This effect is considerably weakened by the introduction of (OH)₂, since resorcin even in very high concentrations did not give sufficient precipitation to cause solidification, an effect which phenol produced in much lower concentration. This difference is also shown by the fact that in very low concentrations resorcin showed retardation against incomplete coagulation of phenol for the same concentration. It should also be noted that phenol when used in solution is more effective than when used in pure form.

2. Comparing benzoic and cinnamic acids it is seen that both retard coagulation, but the action of the latter is far more marked than that of the former. Here too, probably, one of the causes of the difference may lie in their different solubilities, cinnamic acid being the less soluble.

3. However, that solubility alone cannot account for all the effect of a substance on coagulation is seen in the case of benzyl alcohol which, although much more soluble than either of the above acids, has shown interference even in small concentrations.

4. Nitrobenzol and dinitrobenzol have shown rather indifferent effect.

5. Phenylhydrazine gave an almost instantaneous solidification in whatever concentration used. The effect of pyridine was somewhat less marked, requiring higher concentrations and more time for solidification; while the quinoline precipitate remained fluid in high concentrations. In the case of these three substances, the difference in action can hardly be explained by differences in solubilities since pyridine is very soluble, quinoline somewhat less, while phenylhydrazine is least of all.

Antipyrine and caffeine have both definitely retarded coagulation, although neither produced any visible change in plasma. Tyramine was practically without any effect.

Group III. Alkaloids

1. *Quinine alkaloid, pure.* On mixing, all of the quinine came to the top. Clots were all normal in consistency, nor was there any difference in coagulation time as compared with control.

2. *Quinine bisulphate.* On the addition of the salt, there was an almost instantaneous precipitation and solidification. The 2 per cent precipitate soon settled to the bottom, leaving a fluid above; the 4 per cent formed a solid precipitate throughout.

3. *Strychnine alkaloid, pure.* On mixing, the tubes became cloudy; later there was a settlement at the bottom in proportion to concentration. In time and consistency the clots were practically normal.

4. *Atropin alkaloid, pure.* The 0.2 per cent formed a solid scum which on inverting would prevent flowing out, though the rest of the tube remained fluid for a considerable time. Noted to be completely solid after a week. Clot normal, somewhat softer. The 0.4 per cent showed considerable retardation.

5. *Nicotine.* The 2.4 per cent was still fluid after 17 hours and but partly solidified in a week. The rest of the tubes showed marked retardation in coagulation, which was inversely proportional to concentration. The clots were very soft, mushy, with no body, and but little fluid could be expressed. No precipitate was visible.

Of the alkaloids used quinine and strychnine showed themselves to be without any effect on coagulation of plasma; atropin showed some retardation while nicotine gave most definite and marked retardation. Here the difference in action lies perhaps both in the different solubilities and alkalinities since quinine and strychnine show least of these properties; atropine somewhat more and nicotine most of all.

Quinine bisulphate must, of course, be interpreted in terms of its acid content which interfere with coagulation by precipitation.

Group IV. Inorganic substances

1. *Ammonia.* On the addition of ammonia, all tubes showed a hazy cloudiness, which within an hour settled to the bottom as a very light precipitate,⁵ the amount of the latter was in direct proportion to concentration. The supernatant liquid remained permanently fluid.

⁵ This precipitate was tested and was shown to have the following properties: It was insoluble in water and alkalies and in 80 per cent alcohol; soluble in acids and may be reprecipitated by alkalies; not coagulated by heat in acid solutions. Hence the substance is very likely a metaprotein compound of ammonia.

2. *Sodium carbonate.* All tubes gave a whitish precipitate. After 4 hours all were still fluid and somewhat gelatinous in appearance. The 0.1 per cent was found to have become solid in 18 hours; the 0.25 per cent and 0.35 per cent in 5 days, the rest remaining fluid.

3. *Hydrochloric acid.* On the addition of the acid all tubes became immediately cloudy with the formation of a precipitate, the amount of the latter being in proportion to concentration. The 2 per cent and 2.3 per cent were practically all precipitate. After 18 hours the 0.2 per cent and 0.4 per cent were still fluid; the rest of the tubes apparently solid, but the solidity easily disturbed by moderate shaking.

4. *Sulphuric acid.* On the addition of the acid all tubes became cloudy, some separating later a white precipitate. The 0.2 per cent and 0.3 per cent were found solid after 3 days and after 45 minutes respectively; the rest, 0.4 per cent to 1.25 per cent formed thick viscous fluids which later solidified, the solid being very soft and resembling more a precipitate.

5. *Phosphoric acid.* All tubes were still fluid after 15 hours; no precipitate visible. They became solid after 10 days, the clot being soft, with no fluid expressible.

In this group, ammonia and HCl have shown marked interference even in small concentrations; Na_2CO_3 and H_2SO_4 showed both retardation and interference, while H_3PO_4 showed the least effect, producing retardation even in high concentrations.

If we now sum up the effect of various chemicals on coagulation of citrated plasma, we may offer provisionally the following grouping.

I. *No effect.* 1. Some substances such as alkaloids, dinitrobenzol, tyramine hydrochloride, etc., have no effect on coagulation in whatever concentrations used. They do not produce any visible change in the plasma, although some may produce such change (nitrobenzol).

2. Some substances may have no effect in certain concentrations, while producing a definite effect in other concentrations (retardation, etc.) as glycerine, alcohol, etc.

II. *Retardation.* The effect of retardation may show itself either in prolongation of coagulation time or incompleteness of the process.

1. Prolongation of coagulation time varies considerably with each substance and concentration. In most instances, the degree of retardation was directly proportional to concentration used. In some cases, however, notably with antipyrine and nicotine, this was inversely proportional to concentration; the proportional decrease of retardation with increased concentration may be so progressive that the coagu-

lation time will finally fall below that of the control and thus assume the form of hastening. In yet another case (formic acid) retardation started in lower concentrations in direct proportion to concentration, ending with apparent hastening in higher concentration.

Although some substances do not show any other effect but that of retardation (formaldehyde, etc.), other substances will retard in some concentration while producing a different effect in other concentration (glycerine, resorcin, Na_2CO_3 , etc.), most frequently incompleteness or interference with coagulation.

2. Incomplete coagulation. This is not as frequent as the preceding but there are several chemicals which sometimes effect only partial coagulation. As a rule it is accompanied by other effects such as retardation (ether, chloroform, urea, etc.) or precipitation (phenol), and a visible change. From the fact that some cases of incomplete coagulation finally coagulate after a lapse of considerable time, we may regard incomplete coagulation as the next step of a markedly retarded coagulation.

III. Interference. Interference with coagulation may manifest itself in several ways.

1. Precipitation. This is the most common occurrence, the amount of precipitation varying widely with different substances and concentrations used. Thus the members of the saturated fatty acid series, as well as phenylhydrazine, pyridine, HCl , Na_2CO_3 , NH_3 , benzoic acid, etc., will interfere with coagulation even in very small concentrations; other substances (resorcin, etc.) may require somewhat higher concentrations, while still others (acetone, urea, etc.) require relatively high concentrations to produce the same effect.

2. Emulsification. This is of somewhat less frequent occurrence than the preceding and is noted in such substances as oleic acid, benzol, aniline; others (benzaldehyde, acetone, etc.) show a mixed effect. Both precipitation and emulsification are frequently accompanied by retardation or incomplete coagulation.

3. Inhibition. Such substances as nicotine, antipyrine, caffeine, etc., occasionally show apparent inhibitory effects, i.e., no coagulation takes place and no change in the plasma is visible. It usually accompanies retardation and as a factor it probably stands between retardation and interference. To this group probably belong also chloroform and ether.

IV. Acceleration. We have not encountered a single case of genuine hastening of coagulation. Some substances do hasten the process

TABLE 1
Showing the effect of chemicals on coagulation

	NO EFFECT		RETARDATION				INTERFERENCE			ACCELERATION	
	Per cent used	Change in plasma	Per cent used		Degree of retardation	Change in plasma	Per cent used		Emulsion	Per cent used	Change in plasma
			Prolongation	Incompleteness			Inhibition	Precipitation			
Group I. Aliphatic series											
Formic acid.....			0.82-3.8		4.0-0.2					7.3-12.5	Ppt?
Acetic acid.....								0.8-3.9		7.3-12.5	Ppt?
Propionic acid.....								0.83-4.0		7.7-17.2	Ppt?
Butyric acid.....								0.83-7.5			
Valeric acid.....								0.4-7.7			
Oleic acid.....											
Formaldehyde.....											
Alcohol.....	7.7-14.3	Cloudy	3.1-10.0		2.180			20.0-25.0	7.7-25.0		
Glycerine.....	4.0-7.5		0.85-3.0								
Ether.....			7.5-29.0								
			7.5-14.3	20.0-29.0	1.8-2.7						
Acetone.....			4.0-14.3		6.3-9.4	Trans-parent					
Urea.....			7.5	12.5	3.3	Turbid		20.0-29.0		0.8-16.0?	
Hexamethylamine...	0.08-20.0	Settlement									
Hexamethylamine phosphoric acid....			2.0-14.7		200.0						
Choline HCl.....			0.77-3.0	5.0	1.5-4.2						
Glycecol.....			0.3	0.6	220.0	Cloudy	5.0				
Chloroform.....			7.7-14.3	20.0-25.0	1.7-8.0			1.0-10.0			

Group II. Aromatic series

Benzol.....	0.4	Cloudy	0.2-0.8	1.7-1.3		2.0-8.5	13.3-44.0
Phenol.....				3.0		2.0-25.0	
Resorcin.....				1.7-8.0	Turbid Ppt.		1.6-20.0
Benzaldehyde.....							
Benzoic acid.....							
Benzyl alcohol.....							
Nitrobenzol.....	7.7-25.0	Ppt.				0.83-7.7	
Dinitrobenzol.....	0.83-2.4			1.0-330.0			
Cinnamic acid.....	0.83			1.4			
Aniline.....							
Phenylhydrazine.....						7.7-25.0	1.6-20.0
Pyridine.....						4.0-29.0	
Quinoline.....						2.0-29.0	
Tyramine HCl.....	0.08-0.5			130-0.12			
Antipyrine.....				1.5-150.0			20.0
Caffeine.....					4.0		7.7

Group III. Alkaloids

Quinine.....	0.4-4.0						
Strychnine.....	0.2-1.6						
Quinine bisulphate.....						2.0-4.0	
Atropin.....	0.2-0.4			1680-1.5			
Nicotine.....	7.7-29.4		2.4	12.0-1.3			

Group IV. Inorganic chemicals

Ammonia.....						1.2-7.5	
Sodium carbonate.....				200-1030	Ppt.	0.4-0.45	
HCl.....	0.14-0.33					0.21-2.3	
H ₂ SO ₄	0.2-0.3					0.4-1.25	
Phosphoric acid.....	2.0-14.7			480	Ppt.		

more than the controls (phenylhydrazine, pyridine, etc.) but the character of the solid formed resembles more a precipitate than a clot, although no distinct precipitate is visible. It is possible that, as in case of interference, there is more than one mechanism by which coagulation is brought about.

It should be said here that some substances belong properly to this group although not causing any visible change (formic acid, formaldehyde, etc.). There are good reasons to believe that although no change is visible, their apparent retardation is really an interference.

It seems thus quite clear from the above considerations that a close relation exists between the various factors discussed. Thus, substances having no effect on coagulation of plasma in certain concentrations will, in other concentrations, retard the process; while substances retarding in some concentrations will, in higher concentrations, interfere or inhibit. These phases are apparently intimately related, one often passing insensibly into the other, forming a progressive chain of events (no effect—retardation—incomplete coagulation—inhibition—interference) and all probably operating on the basis of some common property.

What then are the conditions that will determine the particular effect of a substance upon coagulation of plasma?

The substances having no appreciable effect on coagulation may act in a particular manner because of one or more of the following reasons.

1. They are not soluble in plasma (alkaloids, dinitrobenzol).
2. They may be soluble in water, but the medium is not favorable for their action (formin requires an acid medium).
3. Their effect is neutralized by an opposite property (tyramine HCl-acid-alkaline-neutral.)
4. The changes produced do not sufficiently alter the plasma so as to interfere with coagulation.

As we pass to the next phase, that of retardation, it would seem that the retarding substance induces certain definite changes in the plasma and these may be due to any one or more of the following conditions:

1. The substances are not soluble in plasma.
2. They do not react chemically with plasma nor form easily a physical mixture (chloroform, ether).
3. They act by dehydration, absorbing water from plasma (glycerine).
4. Their acid or alkaline reaction (caffeine, antipyrine, nicotine, urea, Na_2CO_3).

5. Their effects neutralized by an opposite property, cholin HCl.
6. May be strong reducing agent (formaldehyde).

The same mechanism is probably at work in case of interference, only the changes are so pronounced as to interfere entirely with the process of coagulation:

1. Many of the substances are quite soluble and, uniting with some substances of the plasma, cause precipitation (phenol, resorcin).
2. Their reaction is markedly acid or alkaline (the fatty acids, HCl, benzoic acid, pyridine, ammonia, Na_2CO_3 , etc.). Here evidently solubility in water as such does not play an important rôle, for the effect is the same whether the substances are very soluble in water—as pyridine, HCl, etc.—or increasingly less soluble—as the fatty acids.
3. They may be insoluble in water but form an emulsion, thus depriving the plasma of water (oleic acid, benzol, etc.).
4. They may have a marked solvent or precipitant action on some plasma constituent (alcohol, acetone).
5. Being neither soluble in plasma nor active chemically, their mere presence inhibits coagulation by preventing the aggregation of fibrin threads and crystals into gel formation (chloroform, ether).

That the precipitate is most likely a new chemical compound is readily seen from a qualitative analysis of one of the precipitates (ammonia q.v.); a further analysis of each individual precipitate formed would probably show that the precipitate formed in each case is different. Protein behaves in an acid solution like a cation, and anions render it insoluble; in an alkaline medium, it behaves like an anion, migrating to the anode, and cations render it insoluble. Although in general the rate of precipitation is proportional, *ceteris paribus*, to the molecular conductivity of the added salt, it would seem that while it is true for the inorganic acids, it is not exactly true for the fatty acids, for their ionization decreases as we go up the scale while the precipitation increases at the same time (2).

The process of emulsification is a much simpler one and the disturbance is more of a physical than of a chemical nature.

As it has been observed in numerous instances that the reaction between the substance used and the plasma is a quantitative one, the degree of reaction will obviously depend partly on the properties of the substance, and on the condition of the plasma. It is quite evident that the more soluble a substance is, the less likely it is, *ceteris paribus*, to interfere with coagulation. Thus, the precipitating action of the fatty acids rises as we ascend the scale, while their solubility

decreases at the same time. Phenol in solution interferes with coagulation much less than when used in crystal form, while resorcin interferes less than phenol. Quinine and strychnine are insoluble and have no effect. Finally, it may be said that in general the substances belonging to the second group are considerably more soluble than those of the third. This, perhaps, will explain why a substance will interfere in higher concentrations while only retarding in lower concentrations, since smaller quantities are more easily dissolved. On the other hand, that water per se is not the determining factor is quite evident from the consideration that some substances interfere markedly with coagulation, although they are very soluble (phenylhydrazine, urea, pyridine, etc.).

It has long been observed that the reaction of the blood has a considerable effect on its coagulability. An increased acidity leads to an increased aggregation and finally precipitation of colloidal particles of fibrin, and similarly increased alkalinity may in smaller concentration change the form of the clot from a crystalline form to a structureless mass, and in higher concentrations cause a total failure of clotting (3).

The present work abundantly verifies these observations. Substances having a distinctly acid or alkaline reaction have, in all cases, failed to cause normal clotting, even when used in smaller concentrations. If the alkaloids are cited as exceptions, it should be remembered that those that had no effect on clotting are totally insoluble (quinine and strychnine) while the more soluble ones had a distinct effect (atropine and nicotine). It is possible that the manner in which acids or alkalis interfere with coagulation is in a way comparable to coagulation of protein by heat, since coagulation of blood is due to the formation of an insoluble fibrin compound. According to Chick and Martin (4), in heat coagulation of protein there is first a denaturation or reaction between the protein and hot water, and second, agglutination or separation of the altered protein in a particulate form, the reaction velocity increasing with an increase in acidity or alkalinity. From the purely physical point of view the addition of acids or alkaline ought to disturb the plasma equilibrium since protein salts have a greater attraction for water than electrically neutral protein and, according to Fisher (5), the presence of acid or alkali greatly increases the power of protein to imbibe water. The semi-solid character of some of the clots and the inability to express water from them is probably due to absorption of water by fibrinogen and hence may be regarded as an incomplete clot.

It is also a common observation in the laboratory that old poorly-preserved kephalin loses its thromboplastic properties and may even retard coagulation; and according to McLean (6) the loss of thromboplastic power goes hand in hand with the development of acid reaction. According to W. H. Heard (7) certain concentrations of alkaline earth cause marked retardations of coagulation. It is conceivable that these variations may be accounted for by variations in their respective hydroxyl ions.

Whether certain chemical groupings have a more intimate relation to coagulation of plasma than others, cannot be said definitely. The introduction of either H or OH ions, as stated above, definitely interferes with coagulation; the introduction of the phenolic OH seemingly has a favorable effect on coagulation; while the presence of nitrogen group by effecting a change in the reaction of the substance, interferes with the process.

SUMMARY

The effect of various chemicals on coagulation of citrated plasma has been studied and it has been shown that a chemical may have one of the following effects on plasma.

1. No effect.
2. Retardation of the process by:
 - a. Prolongation of the coagulation time.
 - b. Incomplete or partial coagulation.
3. Interference by:
 - a. Inhibition.
 - b. Precipitation.
 - c. Emulsification.
4. Acceleration.

Reasons have been advanced to show that an intimate relation exists between the factors mentioned and by gradations one may pass into another, suggesting that they all probably work by reason of some common mechanism.

The various properties which may be responsible for the particular effect a substance will have on coagulation have been considered and it was suggested that such effect may depend on the solubility of the substance in plasma, its alkaline or acid reaction, on its dehydrating power, reducing power, etc.

It has also been observed that in the interaction between the chemical and the plasma, new chemical compounds are formed, and the relation is probably a quantitative one.

It is a pleasure to express my appreciation of the encouragement given and valuable suggestions received from Dr. A. D. Hirschfelder. I also wish to thank Dr. E. D. Brown for helpful suggestions, and Mr. J. Paul Quigley, Teaching Fellow in Pharmacology, for assistance in the experiments.

BIBLIOGRAPHY

- (1) WRIGHT: Brit. Med. Journ., 1891, ii, 641.
- (2) ROBERTSON: Journ. Biol. Chem., 1911, ix, 303.
- (3) HOWELL: This Journal, 1916, xl, 527.
- (4) CHICK AND MARTIN: Journ. Physiol., 1912, xlv, 612.
- (5) FISHER: Edema, New York and London, 1915, 22.
- (6) McLEAN: This Journal, 1919, xliii, 586.
- (7) HEARD: Journ. Physiol., 1917, li, 295.

THE INFLUENCE OF PITUITARY EXTRACTS ON THE ABSORPTION OF WATER FROM THE SMALL INTESTINE

MAURICE H. REES

From the Physiological Laboratory of University of South Dakota

Received for publication May 12, 1920

Recent investigations in both the experimental and in the clinical fields indicate quite conclusively that pituitary extracts produce at least a temporary (seven to eight hours) antidiuretic effect when administered subcutaneously. The question arises as to how this antidiuretic action is brought about. Is it a direct or an indirect action on kidney excretion?

Motzfeldt (1) concludes from his experiments on rabbits that pituitary extracts produce an antidiuretic action by stimulating the sympathetic nervous system and bringing about a vasoconstriction within the kidney.

Dale (2) working with perfused kidneys of the dog and the cat, found that pituitary extracts caused a vasoconstriction of the renal vessels. Houghton and Merrill (3) arrived at a similar conclusion. On the other hand, King and Stoland (4) found a vasodilatation of the renal vessels and an increased flow of urine.

The literature regarding the effect of pituitary extracts on the intestine is rather contradictory. Fodera and Pittau (5) in 1909 noted that intravenous injections caused defecation. Increased peristaltic waves following intravenous injections were noted by Bell (6) and by Ott and Scott (7). Shamoff (8), working on isolated loops of the rabbit's intestine, found that posterior lobe extracts gave a relaxation of the intestine.

The writer suggested in previous work (9) that the antidiuretic action of pituitary extracts may be due to an interference with the absorption of water from the intestine. It was noted that rabbits quickly developed a diarrhea following subcutaneous injection of pituitary extracts. Cats showed a marked tendency to vomit following similar injections. These observations suggested the advisability of investigating the

effect of pituitary extracts on the absorption rate of the intestine and also on the emptying time of the stomach.

In the present work we have attempted to find out whether subcutaneous injections of pituitary extracts cause any variation in the rate of water absorption from the small bowel.

METHODS AND RESULTS

Dogs and cats were used in our experiments. One commercial pituitary extract was used, namely, pituitrin (Parke, Davis & Company).

The injections of pituitrin were subcutaneous in every case, and were given four to five minutes before the beginning of the test experiments, that is, at the close of the control period.

In the experiments recorded in tables 1, 2 and 4 the animals were kept under an anesthetic (ether) during the entire experiment.

The small bowel was exposed with as little trauma as possible, and the lumen was washed with warm tap water. A measured amount of warm tap water was then introduced into the cannulated loop of the bowel. At the end of a half-hour period the water remaining in the bowel was removed and measured, and amount of absorption noted. Four or five minutes before the close of this control period the test animals received a subcutaneous injection of pituitrin. The same amount of warm tap water was introduced into the loop of bowel at the beginning of the second and at the beginning of the third half-hour periods and the amount of absorption noted in each case.

In table 1 (first period) it will be noted that normal rate of absorption varies widely in different animals. This is probably due in part to the varied lengths of bowel used.

Following the injections of pituitrin there was delayed absorption in all but two of the fourteen dogs experimented upon. In one of these two dogs (no. 10) the amount of pituitrin used was probably too small to be effective. In the other case (no. 8) the intestinal mucosa was found to be greatly inflamed and this may account for the failure of the pituitrin to delay absorption.

With cats the results were not so uniform since only four out of the six experimented upon showed a delayed absorption after pituitrin injections. The intestines of cats are much more susceptible to trauma than are those of dogs. This may have been a factor in the variation.

The question arises as to whether the decreased absorption noted in the second and third half-hour periods may not be due to the continua-

TABLE 1

Summary of experiments on the effect of pituitary extracts on the rate of absorption of water from the small intestine. The second column of the table shows the amount of water injected into the washed bowel at the beginning of each 30-minute period. Dogs were used except in nos. 15 to 20 in which cats were used. The pituitrin was injected subcutaneously at the close of each control period

NUMBER OF EXPERIMENT	AMOUNT OF WATER INJECTED	AMOUNT OF PITUITRIN INJECTED	FIRST PERIOD CONTROL		SECOND PERIOD		THIRD PERIOD	
			Water absorbed		Water absorbed		Water absorbed	
			First loop	Second loop	First loop	Second loop	First loop	Second loop
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
1	250	1.0	200.0	210	160.0	175	155	170
2	250	1.0	120.0	155	115.0	135	100	102
3	300	0.5	255.0	255	235.0	240		
4	200	0.5	100.0	90	70.0	68		
5	100	0.5	60.0	58	50.0	55		
6	200	1.0	125.0		100.0		86	
7	300	1.0	192.0		170.0		120	
8	150	0.5	100.0		105.0			
9	200	0.5	40.0		30.0			
10	100	0.25	62.0		75.0			
11	100	0.5	85.0		18.0			
12	200	1.0	130.0		105.0		100	
13	300	1.0	160.0		135.0		120	
14	300	1.0	170.0		130.0		100	
15	25	0.5	21.5		13.5		12	
16	25	0.5	21.0		14.0		11	
17	50	1.0	31.0		31.5		27	
18	50	1.0	33.5		41		26	
19	50	1.0	12.0		0		7	
20	50	1.0	15.0		6.0		5	

tion of the anesthetic and to the operative procedure. To determine this point we took the normal absorption rate on several animals without pituitrin injections. An inspection of table 2 will show that in the absence of any pituitrin injections the absorption rate may be even greater in the second and third half-hour periods than it is in the first half-hour or control period. In no case was there a marked decrease in the second period, and in only one case (no. 21) was there a marked decrease in the third period.

In order that we might still further rule out the possible effect of the anesthetic we repeated the absorption experiments on four decere-

TABLE 2

Control experiments: To show the normal rate of the absorption of water from the small intestine during the first, second and third half-hour periods of the experiment. Dogs were used in experiments 21, 22 and 23. Cats were used in the remaining experiments

NUMBER OF EXPERIMENT	AMOUNT OF WATER INJECTED	AMOUNT OF WATER ABSORBED PER HALF HOUR		
		First period	Second period	Third period
	cc.	cc.	cc.	cc.
21	200	85.0	88	65.0
22	50	42.0	43	32.0
23	50	42.0	40	28.0
24	25	13.0	15	13.5
25	25	8.0	7	14.0
26	25	12.5	15	10
27	25	9.0	8	10.0

TABLE 3

Showing the rate of water absorption from the small intestine before and after the injection of pituitrin in decerebrated dogs. The dogs were decerebrated three hours previous to the beginning of the experiments on absorption.

NUMBER OF EXPERIMENT	AMOUNT OF WATER INTRODUCED	AMOUNT OF PITUITRIN INJECTED	AMOUNT OF WATER ABSORBED PER HALF HOUR		
			First half-hour period, control	Second half-hour period	Third half-hour period
	cc.	cc.	cc.	cc.	cc.
28	300	0.50	150	135	100
29	300	0.50	135	100	85
30	450	1.00	225	60	
31	200	0.50	125	20	14

TABLE 4

Effect of pituitary extract on the flow of blood from the mesenteric veins; 0.5 cc. of pituitrin was injected subcutaneously in each experiment. Dogs were used

NUMBER OF EXPERIMENT	BEFORE INJECTION OF PITUITRIN, CONTROL	FIVE MINUTES AFTER INJECTION OF PITUITRIN
	gtt. per minute	gtt. per minute
32	30	26
33	160	80
34	70	61
35	140	130

brated dogs. The findings in these experiments are recorded in table 3. In operating on these animals hemorrhage was kept down to a minimum and sufficient time (three hours) was allowed for the animal to recover from the anesthetic and, in so far as possible, from the shock of the operation. It will be noted that in every case there was a decrease in the absorption rate during the second and third periods, that is, following the injection of pituitrin. This shows that the anesthetic could not have been responsible for the decreased absorption following the pituitrin injections.

It was thought that vasoconstriction of the vessels of the intestinal wall might be a factor in reducing the absorption after pituitrin injections. This possibility was investigated by placing a cannula in one of the mesenteric veins, noting the rate of blood flow by the drop method. By referring to table 4 it will be noted that there is a reduction in the number of drops per minute after pituitrin injection. The reduction was not pronounced except in one case (no. 33); in fact, they do not go much beyond the limit of error due to the difficulty of preventing the blood from clotting in the cannula.

DISCUSSION AND CONCLUSIONS

Our investigation leads us to the conclusion that subcutaneous injections of pituitrin bring about a delay in the absorption of water from the small intestine.

This delay does not seem to be sufficient, in most cases, to entirely account for the delay in the excretion of water from the kidneys which has been found to result from pituitrin injections.

It is possible that the subcutaneous injection of pituitrin may cause some vasoconstriction of the intestinal vessels. This can not be pronounced or very extensive since it has been repeatedly shown and was verified by ourselves in this and previous work, that subcutaneous injections of pituitary extracts do not cause a variation in the general blood pressure.

Motzfeldt (1) suggested that the antidiuretic action of pituitary extracts is due to splanchnic stimulation, causing a vasoconstriction within the kidneys. It is possible that this mild splanchnic stimulation also extends to the vessels of the intestine.

BIBLIOGRAPHY

- (1) MOTZFELDT: Journ. Exper. Med., 1917, xxv, 153.
- (2) DALE: Biochem. Journ., 1909, iv, 427.
- (3) HOUGHTON AND MERRILL: Journ. Amer. Med. Assoc., 1908, li, 1849.
- (4) KING AND STOLAND: This Journal, 1913, xxxii, 405.
- (5) FODERÀ AND PITTAU: Arch. Ital. de Biol., 1909, lii, 370.
- (6) BELL: Brit. Med. Journ., 1909, ii, 1609.
- (7) OTT AND SCOTT: Amer. Med., 1911, vi, 154.
- (8) SHAMOFF: This Journal, 1916, xxxix, 268.
- (9) REES: This Journal, 1918, xlv, 471.

THE SPECIFIC INFLUENCE OF THE ACCELERATOR NERVES ON THE DURATION OF VENTRICULAR SYSTOLE

CARL J. WIGGERS AND LOUIS N. KATZ

*From the Physiology Laboratory of Western Reserve University School of Medicine,
Cleveland*

Received for publication May 12, 1920

INTRODUCTION—PREVIOUS WORK

Ever since the discovery of the accelerator nerves in 1867 by v. Bezold and Bever (1) and the Cyon brothers (2), a sporadic interest has been manifested in the question as to whether, in addition to altering the heart rate, these nerves also specifically affect the strength and duration of ventricular contraction. It does not seem to have been realized, however, that a careful study of their effects on the duration of ventricular systole is capable of disclosing whether the normal ventricular beat is controlled entirely in a mechanical way or whether it is also specifically controllable through nervous influences.

The idea that the mammalian ventricle is controlled in a simple mechanical fashion received its greatest support from the oncometer experiments of Henderson and his co-workers (3). They found that, under normal conditions of venous pressure, the volume curves of the ventricles at all heart rates are practically superimposable on portions of a standard curve obtained during a long vagus beat. This led to the formulation of the law of "uniformity of behavior" according to which the systolic volume discharged is entirely a function of the heart rate. Later Henderson and Barringer (4) presented work which indicated that this law also holds when the heart rate is increased by excitation of the accelerator nerves.

Although emphasis has not been specifically laid on the fact by Henderson and his co-workers, it is evidently a corollary of the "uniformity of behavior" law that the duration of systole is fixedly related to the cycle length under all conditions which produce a change in the heart rate. A review of other experimental work indicates, however,

that when the accelerator nerves are stimulated, systole and diastole vary quite independently. Thus Baxt (5) in 1878 reported that stimulation of an accelerator nerve chiefly reduces the phase of systole. His technical procedures were, however, crude and entirely unreliable. Contrary effects on the duration of systole were reported from the stimulation of the vagus nerve by Klug (6) in 1881, and by MacWilliams (7) in 1888. The inability to decide questions of this nature, by their methods, is now obvious. The first experiments, therefore, that today would be regarded as accurate and at all decisive were made by Hürthle (8) in 1891. This investigator recorded the arterial pulse tracings with his membrane manometer and used the interval from the primary rise to the dicrotic notch as an index of the duration of systole. He found that the period of systole, so determined, is slightly abbreviated when cardiac acceleration is induced by stimulation of the accelerator nerves or when the vagi nerves are sectioned. Accelerator nerve stimulation, after sectioning of the vagi nerves, produces a marked decrease in the duration of systole; stimulation of the vagi, on the other hand, affects systole very slightly, but exerts its chief influence on the duration of diastole. In 1897 Frank (9) not only substantiated this work but reported, in addition, that by simultaneous stimulation of the vagi and accelerator nerves with suitably adjusted currents, it is possible to decrease the duration of systole even when the length of the diastolic phase is unaltered. In the comprehensive investigations of the accelerator and vagi nerve action, carried out by Reid Hunt (10) in 1899, can be found, among other data, the following observations: Section of the accelerator nerves causes a prolongation of both systole and diastole, the former being lengthened rather more than the latter. Stimulation of the accelerator nerves causes a shortening of both systole and diastole. Stimulation of a vagus nerve chiefly prolongs diastole, affecting systole relatively little. Under certain conditions, simultaneous stimulation of vagus and accelerator nerves produces a shortening of systole while diastole remains unaffected.

The conclusion that such results are not in accord with a mechanical regulation of the heart beat does not necessarily follow. Since the rate of systolic ejection diminishes toward the end of systole the duration of systole must by the law of "uniformity of behavior" become increasingly abridged as the cycles shorten more and more. Thus, in the volume curve reproduced in figure 1, a reduction in cycle length from 0.8 to 0.7 second entails a reduction in the ejection phase of systole from 0.215 to 0.21 second; while an equivalent reduction in

the cycle length from 0.4 to 0.3 mathematically decreases the ejection phase from 0.175 to 0.15 second. Inasmuch as vagus section and vagus stimulation ordinarily do not alter the heart rate beyond ranges where slight variations might be expected, whereas accelerator stimulation quickens the beat so much that a more pronounced shortening of systole might be anticipated, it follows that *the mere demonstration that accelerator stimulation shortens the systole is proof neither of any specific influence of these nerves over ventricular contraction, nor does it prove that the heart deviates from a mechanical scheme. Only if it can be shown that the periods of systole during accelerator nerve stimulation vary materially from those which may be accounted for on the basis of volume curves, can any inference be drawn as to a selective action of the accelerator nerves on the ventricle.*

METHODS OF INVESTIGATION

In order to determine whether the lengths of systole and diastole during accelerator stimulation conform to or deviate from a mechanical regulation of the normal heart beat, we first established a plot of the theoretical systoles that should obtain at different cycle lengths and then compared, in the form of a plot, the actual systoles at different cycle lengths with these theoretical values.

Experimental procedures. In order to accomplish this it was necessary to determine accurately the duration of systole and diastole while the circulatory conditions were as nearly normal as possible. It was especially important, for example, to avoid opening the chest and the institution of artificial respiration—events which in themselves alter venous pressure relations considerably.

We therefore determined the systole and cycle lengths by optically recording the heart sounds by means of the direct sound recording capsules of Wiggers and Dean (11). The main vibrations of the first sound correspond to the first rise of intraventricular pressure while the first vibration of the second sound is synchronous with the incisura of the aortic pressure curve, events which mark the onset of systole and diastole respectively (12).

Dogs anesthetized with morphine and chloretone were used as experimental animals. The vagi and accelerator nerves together with the stellate ganglion were first prepared for section and stimulation, the latter being dissected without opening the thorax. The thorax was then shaved and a sound receiver adjusted over the apex region

by an elastic band encircling the thorax. This receiver was connected with the sound recording capsules by a tube having an adjustable lateral opening. The vibrations of a 50 v. d. tuning fork were simultaneously recorded. In order to gauge the appropriate time for taking sound tracings on bromide paper, a carotid-pressure curve was continuously traced on a long paper kymograph.

From the optical records, the lengths of consecutive cycles and corresponding systoles were subsequently determined. This was done in about 3000 cycles recorded during many experiments on ten different dogs.

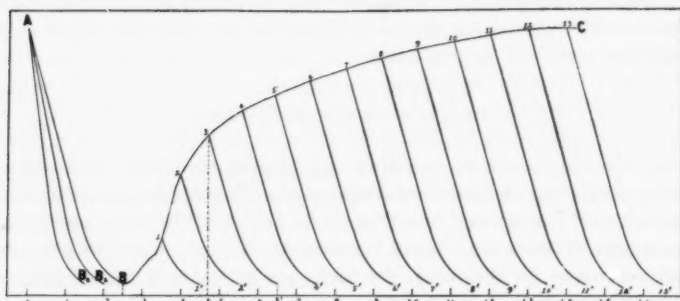


Fig. 1. ($\frac{1}{4}$ original size.) Diagram constructed for experiment C 207, determining the relation of systole and cycle length at various heart rates—also the method of adapting volume curves to vagal beats with different periods of systole. Shows arcs used when ejection phases AB , AB' and AB'' are equal to 0.25, 0.20 and 0.15 second respectively. $B-1'$, $B-2'$, $B-3'$, etc., indicate duration of cycle. $1-1'$, $2-2'$, $3-3'$, etc., the period of systole. Abscissa = 0.1 second. Detailed description in text.

Methods of constructing a curve expressing the theoretical duration of systoles at different cycle lengths. In order to obtain a curve of the theoretical systoles at all heart cycles according to Henderson's mechanical conception of cardiac control, it was first necessary to plot a theoretical volume curve for each animal. This construction, however, is beset with a number of difficulties which, we believe, are not insurmountable. We started out according to a very simple plan: On large sized coördinate paper, a volume curve similar to that plotted as a standard by Henderson (13) was laid off. A reduced reproduction with coördinates omitted, is shown in figure 1 (A , B , C). At varying points, e.g., at 2, 3, 4, 5, etc., arcs of the standard ejection curve A B

were drawn. The abscissal distances $B-1'$, $B-2'$, etc., then denote the duration of the cycle and the abscissal distances $1-1'$, $2-2'$, etc., measure the corresponding systole lengths. These intervals can obviously be readily and accurately determined on large coördinate paper for a consecutive range of cycles. In figure 1, one such cycle $B-3'$ and its corresponding systole $3-3'$ is indicated by dotted lines. So far the process is not dissimilar to that employed by Henderson except that the relation of systole to each cycle length was determined in cycle lengths differing by 0.1 second. This $\frac{\text{systole}}{\text{cycle}}$ ratio will hereafter be referred to briefly as the s/c ratio.

The data so obtained were plotted by dots on coördinate paper, as shown in figure 4, the ordinates representing the duration of systole, the abscissae, the cycle lengths. By connecting these data by lines a curve s/c is obtained from which the theoretical s/c ratio at any heart rate can be derived.

It soon became obvious, however, that such a theoretical curve of s/c ratios could not be applied to different animals, inasmuch as certain variable factors were not taken account of. *In the first place* we found that the duration of long vagal systoles varied from 0.22 to 0.32 second in different animals with corresponding variations at more rapid rates. It therefore became necessary to construct for each animal a separate hypothetical volume curve based on its vagal systole and from it to derive a curve of s/c ratios applicable to that animal.

This we did after the following manner: The duration of systole was first determined during a long vagus beat occurring after slowing had been established for some time. Inasmuch as only the interval of systolic ejection and not the total period of systole is concerned in the construction of the volume curve, an interval of 0.05 second was deducted from the vagal systole for the isometric period.¹

The resulting interval of systolic ejection was then laid off on the abscissae of large-sized coördinate paper and an arc having the same contour as that given in Henderson's standard curve was drawn to fill this time. Thus, in figure 1, the arcs AB , AB^1 and AB^2 have the same

¹ This we believe to be allowable for, according to the investigations of Hürthle (8), de Heer (14), Garten (15) and Frank (16), this is an average period which is not affected in length by such changes in the circulation as occurred during the course of our experimentation. Even if this period does vary slightly in different dogs, no significant error can be introduced since the same figure was again added before the plot of s/c ratios was made.

conformation but correspond to ejection periods of 0.25, 0.20 and 0.15 second, respectively. In this particular instance, the arc AB , with a duration of 0.25 second was subsequently used as a pattern for the smaller segments $1-1'$, $2-2'$, $3-3'$, etc. It is obvious that if the systolic ejection time were either 0.15 or 0.20 second, the arcs AB' or AB^2 must be used as a pattern for the smaller segments.

The further criticism may be anticipated that belief in a "uniformity of behavior" law does not necessitate the assumption that hearts of different animals with the same systole lengths necessarily have the same contour of ejection curve. According to Henderson's (4) results, however, volume curves taken under normal conditions show that the

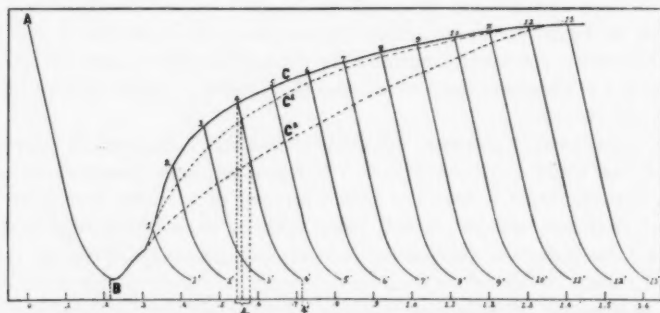


Fig. 2. ($\frac{1}{4}$ original size.) Diagram constructed for experiment C 210, showing volume curve with ejection phase of 0.215 second and three possible diastolic filling curves C , C' and C'' . The effect of diastolic filling rate on the length of systole is indicated by the systoles $4-4'$. Abscissa = 0.1 second. Detailed description in text.

curve of systolic discharge is a straight line at all points except at the extreme lower end. A variation which could occur only at this place would therefore affect the duration of only the very shortest cycles, e.g., $1-1'$ in figure 1. Since cycles of such short duration were never obtainable in any of our experiments, however, it is clear that this criticism is of no practical importance.

A second practical difficulty arose, however, in the correct projection of the diastolic filling curve. Even though all possible precautions were taken to maintain an effective venous pressure sufficient, according to Henderson and Barringer (4), to insure maximal filling, it would not be in disagreement with the idea of superimposable beats to suppose that the filling curves of different animals are dissimilar. Indeed

it is quite conceivable that the heart within the intact thorax has a filling curve quite different from any capable of registration by oncometric methods. Such differences in filling, however, will affect considerably the relation of systole to cycle length at different heart rates. This is illustrated in figure 2 where one may suppose the heart to be filled according to any of three curves C , C' or C^2 . Reference to the dotted lines bracketed as 4 in figure 2 or to the three corresponding plotted curves of s/c ratios in figure 3, indicates that the theoretical curves depend fundamentally on the unknown character of the filling curve.

Confronted with the necessity of having some gauge as to the type of filling occurring in each animal's heart, we selected that line of s/c ratios which most nearly coincided with the long vagal beats and the normal beats of the animal.

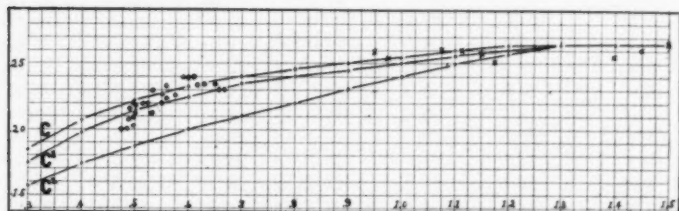


Fig. 3. Three curves showing relation of systole to cycle length at different heart rates when the rate of diastolic filling differs. Constructed from data of table 1 and volume curves of figure 2. C , C' and C^2 are corresponding curves. Abscissae represent cycle length; ordinates, systole lengths, in seconds. Circles, normal s/c ratios; crosses, s/c ratios of vagal beats. Detailed description in text.

The entire method of deriving our theoretical curve of s/c ratios, thus outlined in principle, may be further clarified by following the consecutive steps in a typical experiment:

In experiment C 210, stimulation of the right vagus caused a slowing of the ventricles from which a cycle having a period of 1.55 second and a systolic period of 0.265 second was selected. Deducting 0.05 second for the probable isometric interval, leaves an ejection phase of 0.215 second. This distance is laid off on coördinate paper and an arc AB drawn. A reduced figure with coördinates omitted for reasons pertaining to reproduction is shown in figure 2. In this case, three possible filling curves, C , C' and C^2 are drawn. By inscribing arcs of the ejection curve AB , at intervals of 0.1 second from points 1, 2, 3, 4

and so forth, and measuring the horizontal distances between 1-1', 2-2', 3-3', for each type of filling curve (illustrated by dotted lines bracketed as 4) three possible durations of the ejection phase for each cycle length are obtained. Now adding again 0.05 second previously deducted gives the theoretical systoles for each cycle under three conditions of ventricular filling. This is shown in the following table:

TABLE 1

DURATION OF CYCLE	DURATION OF THEORETICAL EJECTION PHASE IN FIGURE 2			DURATION OF TOTAL SYSTOLE ACCORDING TO FIGURE 3		
	Curve C	Curve C'	Curve C ²	Curve C	Curve C'	Curve C ²
0.2	9.0	9.0	8.5	14.0	14.0	13.5
0.3	13.5	12.5	10.75	18.5	17.5	15.75
0.4	15.75	14.5	12.0	20.75	19.5	17.0
0.5	17.25	16.5	13.75	22.25	21.5	18.75
0.6	18.0	17.5	15.0	23.0	22.5	20.0
0.7	19.0	18.5	16.0	24.0	23.5	21.0
0.8	19.5	19.0	17.0	24.5	24.0	22.0
0.9	20.0	19.5	18.0	25.0	24.5	23.0
1.0	20.5	20.0	19.0	25.5	25.0	24.0
1.1	21.0	20.5	20.0	26.0	25.5	25.0
1.2	21.5	21.0	20.7	26.5	26.0	25.7
1.3	21.5	21.5	21.25	26.5	26.5	26.25
1.4	21.5	21.5	21.5	26.5	26.5	26.5
1.5	21.5	21.5	21.5	26.5	26.5	26.5

Plotting these theoretical systoles in relation to the cycle, as in figure 3, the three curves C, C' and C² are obtained. If now we plot as small circles the actual s/c ratios found during the natural heart cycles of the animal, it will be seen that they follow the line C' most exactly. This line is therefore adopted as the theoretical line of s/c ratios for other comparisons. In a similar way, a line of s/c ratios was derived for all experiments and in the rest of the plots the line selected is alone reproduced.

It is of interest to add that, according to these analyses, we found that the hearts of our animals followed a type of filling not dissimilar to that described by Henderson as typical for the normal heart. In this instance we believe, for example, that the volume curve of the ventricle corresponds to the line C' in figure 2.

EXPERIMENTAL RESULTS

Comparison of the actual s/c ratios during accelerator nerve stimulation with the theoretical values at corresponding heart rates. After the theoretical curve of s/c ratios had been thus determined for each animal, we plotted the actual s/c ratios obtained under different experimental conditions in relation to it, especial attention, of course, being directed to the influence of the accelerator nerves. As the results require detailed presentation, an analysis of three experiments, typical of all cases, is appended.

Experiment C 207 (fig. 4). The theoretical s/c ratio curve selected as applying to this animal's heart is shown as a solid line (s/c) connecting dots at 0.1 second intervals. The small circles close to the line

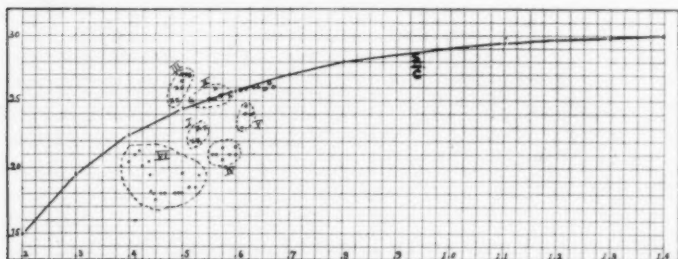


Fig. 4. Plot from experiment C 207, showing relation of actual s/c ratios to the theoretical curve. Abscissae, cycle lengths; ordinates, systole lengths, in seconds. Detailed description in text.

show normal s/c relations. The right stellate ganglion was stimulated first, but we shall defer the discussion of this effect. Then both vagi nerves were sectioned. The s/c ratios of 13 measured cycles are indicated by crosses of group I. It is evident that when vagus control is suddenly removed and the accelerator control alone remains, the s/c ratio is below that of the theoretical line. Within a few minutes, however, the duration of systole increases again, as shown by the crosses of group II, which represent measurements of 13 cycles ten minutes after vagotomy. The left vagus nerve was then stimulated but, with the exception of a few beats, the cycles were so long that they were omitted from this plot for reasons of reproduction. It may be noted, however, that the systoles following immediately after stimulation began were slightly below the theoretical line, while those occur-

ring later coincided with it. Immediately following this stimulation, 12 measured cycles showed s/c ratios represented by the crosses of group III. The obvious after-effect seems to be a slight increase in the heart rate during which the systole lengths are somewhat longer than the theoretical curve calls for. Such results indicate that perhaps vagus stimulation is not entirely without an influence on ventricular systole,—whether direct or indirect we are not able to say. A detailed discussion of this question is, however, not within the province of the present communication.

We may now return to the effects of accelerator nerve stimulation. The dots of group IV represent the s/c ratios of 17 measured cycles recorded during accelerator stimulation while the vagus nerves were

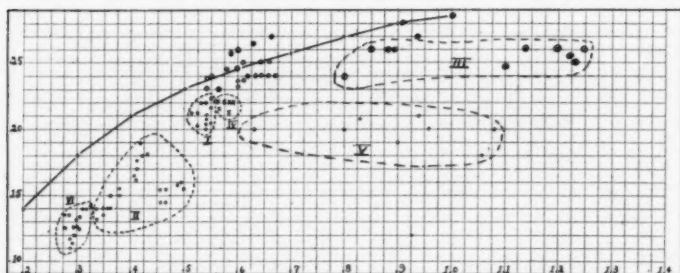


Fig. 5. Plot of data from experiment C 209, showing relation of actual s/c ratios to theoretical curve. Abscissae, cycle lengths; ordinates, systole lengths in seconds. Description in text.

still intact. The dots of group V show the s/c ratios of 14 measurements shortly after the cessation of stimulation. The dots of group VI, representing 32 measured cycles indicate the greater effect of accelerator stimulation when the vagi nerves had been divided.

The data plotted in relation to the theoretical s/c ratios make it obvious without further comment that the stimulation of the accelerator nerves reduces systole a great deal more than can be accounted for by a mechanical abbreviation of the systolic portion of the volume curve.

Experiment C 209 (fig. 5). The line of theoretical s/c ratios in this experiment was derived from a vagus beat having a cycle length of 0.98 seconds and a systole length of 0.285. The small circles arranging themselves around the theoretical line are normal cycles representative

of 40 measured beats. The large dots represent ratios occurring during vagus excitation. The small dots of group *I* indicate the duration of systole during mild stimulation of the stellate ganglion; those of group *II*, the effect of exciting the ganglion with a stronger current. In both instances the vagus nerves were intact. It is evident that a reduction of s/c ratios below the theoretical value occurs.

In order to determine the effect of accelerator stimulation on the s/c ratio when cycles of longer duration occurred, we tested the effect of simultaneous stimulation of the accelerator and vagus nerves. This could, of course, be accomplished by simultaneous electrical stimulation of these nerves. We found it, however, more expedient to induce the vagus stimulation through chemical means. Pituitary extract when injected, produces such a stimulation in some animals as was fortunately the case in this experiment.

The dotted circles of group *III*, arranging themselves somewhat below the theoretical line, show the duration of systole during such pituitary slowing. That this slowing is at least predominantly due to vagus stimulation, is evidenced by the fact that subsequent section of the vagi restores the normal rate and duration of systole. This is shown by the crosses of group *IV* which represent the systoles of 18 measured cycles after the vagi nerves were cut and while pituitrin was still acting.

While the heart rate remained slow due to the pituitary extract, the stellate ganglion was again stimulated. Although this caused some increase in rate, the heart rate did not equal that natural to the animal. The s/c ratio decreased not only far below the theoretical ratios for such cycles, but also far below the s/c ratios of other much shorter cycles. This is evident on comparing the dots of group *V*, representing 9 measurements of beats occurring during accelerator nerve stimulation, either with the dots of group *I*, with the crosses of group *IV* or with the circles representing normal s/c ratios. Finally, after the vagi had been divided and s/c ratios represented by the crosses of group *IV* had been attained, the stellate ganglion was again stimulated. The results of 27 measurements are shown by the dots of group *VI*.

These results indicate clearly that the s/c ratio is much reduced below the theoretical expectations by the influence of the accelerator nerves, not only at rapid, but at slower heart rates as well.

Experiment C 210 (fig. 6). In this experiment the curve of the theoretical s/c ratio was derived as analyzed in detail in an earlier portion of the paper. The small circles represent the actual s/c ratios of 60

normal beats. The small circles in group *III* are representative of cycles following the injection of $1/6$ grain of atropine sulphate.

The dotted circles show the s/c ratios of 13 cycles obtained during the action of pituitary extract early in the experiment. Section of the vagi abolished this slowing and established a normal rate. Seven measurements of right vagus and 18 observations of left vagus stimulation are shown by crosses. Under all of these conditions of varying vagus activity the s/c ratio does not deviate in any pronounced fashion from the theoretical line.

On accelerator nerve stimulation, this conformity is no longer observed. The dots of group *I* represent measurements of 53 cycles during and immediately after accelerator nerve stimulation. The dots of group *II* show the results of 33 measurements of cycles obtained

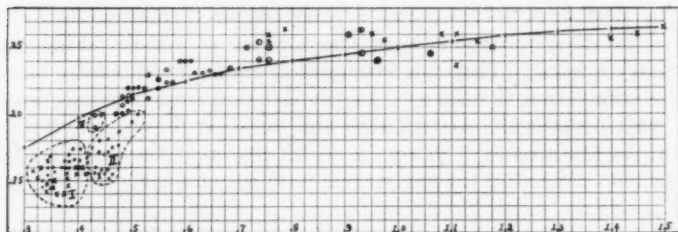


Fig. 6. Plot of data from from experiment C 210, showing relation of actual s/c ratios to theoretical curve. Abscissae, cycle lengths; ordinates, systole lengths, in seconds. Description in text.

while the accelerator nerves were stimulated and the heart slowed by pituitary extract. With the exception of three dots in this group which, as a matter of fact, correspond to cycles at the very onset of stimulation, the s/c ratios are far below the theoretical line.

Finally, when the vagi nerves had been sectioned, 3 cc. of a 1:50,000 solution of epinephrin, which presumably also affects the sympathetic endings in the heart, were injected. The crosses included in group *I* illustrate the s/c ratio of 44 rapid beats thus produced. It is obvious that epinephrin like accelerator stimulation acts to abbreviate systole much more than can be explained by a mechanical shortening of systole at these rates.

The mechanism of accelerator action. Discussion of results. The experiments above cited indicate clearly that, while under normal

conditions changes in the duration of systole may conform reasonably to the changes anticipated if the ventricle beats according to a uniform plan, the abbreviation of systole induced through accelerator activity both at rapid and slower heart rates is in excess of that which may be accounted for by a mechanical shortening. In some way the accelerator nerves exert an influence on ventricular systole which can be termed specific. Before it may be assumed, however, that such an influence is exerted directly on the ventricular muscle and acts to abbreviate the contraction process, diligent inquiry must be made for the involvement of some possible mechanical mechanism.

The possibility suggests itself at once that the rapid heart action induced by accelerator stimulation causes an increase in the minute volume discharged which might conceivably operate to reduce the venous pressure and auricular filling. If this were the case, the rate of ventricular filling, especially in early diastole, would decrease and this might mechanically abbreviate systole. In other words, it is conceivable that under rapid heart action we would have an entirely different filling curve and that therefore the theoretically derived curve would no longer apply. Unfortunately, we did not follow the venous pressures throughout the experiment nor do such observations during accelerator stimulation seem to have been reported. In the volume curves recorded by Henderson (4) during accelerator nerve stimulation, there is no such indication of reduced filling; indeed the rate of filling appears to us slightly increased. Such an explanation, however, could not account for the greatly abbreviated systoles occurring when the accelerator nerves affect beats maintained at or below the normal rate by simultaneous vagus action.

The further suggestion that the reduction of systole by accelerator stimulation is due to a shortening of the isometric period rather than the ejection phase, might be entertained were the reduction itself not frequently far greater than the entire isometric interval. By no plausible conception at present presenting itself can the effect of the accelerator nerves be explained otherwise than through a specific effect on the duration of muscular contraction itself.

This explanation is further substantiated by the lack of a fixed relation between the duration of systole and diastole when we follow them from beat to beat during increased accelerator activity. Thus, accelerator nerve stimulation usually causes an immediate shortening of diastole while the period of systole shortens gradually and progressively as stimulation continues. In some experiments, systole continues to

shorten even when diastole is again increasing. We have plotted experiments in which, during continued stimulation, diastole actually lengthened again so that its duration was greater than normal, yet systole continued at its shortest length. When stimulation ceases the systolic period usually remains shortened for as much as 20 seconds; a shortened diastole, on the contrary, at once begins to increase in length. The general trend of such experiments is shown in an abbreviated plot in figure 7. At *A*, a few consecutive normal cycles are indicated. At *B*, the 20th and subsequent beats during accelerator stimulation are shown. A moderate decrease in systole and a marked decrease in diastole are evident at this time. As stimulation continues (*B-C*),

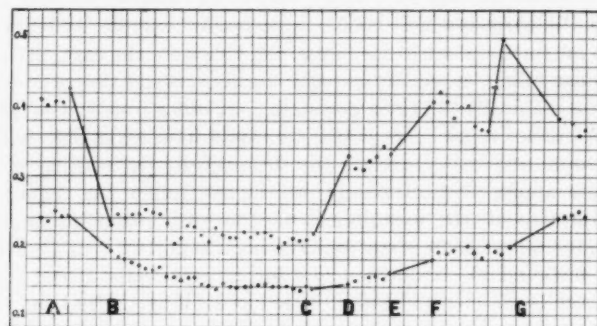


Fig. 7. Plot from experiment C 209 II, showing effect of accelerator nerve stimulation on duration of systole and diastole in consecutive heart beats, each represented by a dot. Upper plot, duration of diastole; lower curve, systole; ordinates represent time in seconds. Description in text.

systole decreases more than diastole. Between *C* and *D*, 25 beats are omitted. While accelerator stimulation continues at *D*, the length of systole remains practically unaltered while the period of diastole has recovered considerably. At *E*, stimulation ceased. Between *E* and *F*, 6 beats were omitted. By this time, *F*, diastole has regained its normal length, but systole continues shortened. *G* is a later normal control.

Another illustration is shown in the case of epinephrin stimulation in figure 8. The plot starts with a few normal control data. At *A*, the systole and diastole after the 20th beat following epinephrin injection are plotted. Both vagi nerves had been severed and a previous dose of $\frac{1}{100}$ grain of atropine sulphate had been administered. In this

instance, diastole first lengthens while systole progressively decreases (A B). Between B and C, 45 beats are omitted and by that time systole has again begun to lengthen while diastole decreases further. This again demonstrates the lack of a fixed relation between systole and diastole when the accelerator endings are stimulated.

In view of these facts and since cardiac acceleration under normal conditions is undoubtedly often due to accelerator nerve activity, the hypothesis that the ventricle normally beats according to a "uniformity of behavior" law at these rapid rates should be submitted to further experimentation.

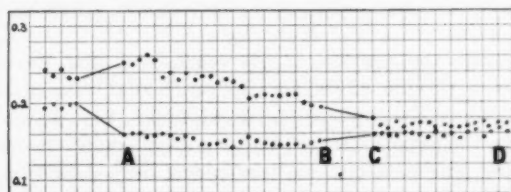


Fig. 8. Plot from experiment C 210 VII, showing effect of epinephrin on duration of systole and diastole in consecutive heart beats, each represented by a dot. Upper plot, duration of diastole; lower curve, systole; ordinates represent time in seconds. Description in text.

SUMMARY AND CONCLUSIONS

Although it had been shown by previous investigators that stimulation of the accelerator nerves causes a marked reduction in the duration of systole, it had not been demonstrated that this reduction was greater than could be accounted for on Henderson's law of "uniformity of behavior," and consequently no clear demonstration existed of any specific effect on the ventricular musculature.

By determining the duration of systole as well as the cycle length in a slow vagal beat, we found it possible to construct a probable volume curve for each animal and from it to derive a plot of the theoretical relation that should exist between cycle and systole lengths at any heart rate if the heart, during changing nervous action, beats according to a uniform law.

The actual systole and cycle lengths were determined from the recorded heart sounds during a wide range of heart rates obtained through vagus sectioning, vagus stimulation and accelerator excitation

and these values were then plotted on coördinate paper in relation to the theoretically constructed curve of $\frac{\text{systole}}{\text{cycle}}$ ratios.

Upon doing this, it was found that while the actual $\frac{\text{systole}}{\text{cycle}}$ ratios obtained during normal conditions and during vagus stimulation coincided reasonably well with the theoretically derived values, *the length of systole during accelerator stimulation and during the action of epinephrin were markedly less than those indicated by the theoretical curve.* Inasmuch as this occurred whether the heart rate was actually increased or maintained slow by the simultaneous action of pituitary extract, it is difficult to refer this to any mechanical effect on the venous pressure and ventricular filling.

Consequently, the conclusions are reached *a*, that the accelerator nerves have a specific effect on the ventricular musculature which operates to reduce the contraction period; and *b*, that, in view of these observations, the hypothesis that under normal conditions the ventricle operates according to a uniform mechanical law, should be subjected to further investigation.

BIBLIOGRAPHY

- (1) V. BEZOLD AND BEVER: Untersuchungen aus d. physiol. laborat. im Würzburg, 1867, ii, 235.
- (2) CYON AND CYON: Centralbl. f. d. med. Wissensch., 1866, 801; Archiv f. Anat. u. Physiol., 1867, 389.
- (3) HENDERSON, ET AL: This Journal, 1906, xvi, 325; 1913, xxxi, 288, 352.
- (4) HENDERSON AND BARRINGER: This Journal, 1913, xxxi, 297.
- (5) BAXT: Arch. f. Physiol., 1878, 122.
- (6) KLUG: Arch. f. Physiol., 1881, 260.
- (7) MACWILLIAMS: Journ. Physiol., 1888, ix, 359.
- (8) HÜRTHLE: Arch. f. d. gesamt. Physiol., 1891, xlv, 89.
- (9) FRANK: Sitzungsber. d. Gesellsch. f. Morph. u. Physiol., München, 1897.
- (10) HUNT: This Journal, 1899, ii, 395.
- (11) WIGGERS AND DEAN: Amer. Journ. Med. Sci., 1917, cliii, 666.
- (12) WIGGERS AND DEAN: This Journal, 1917, xlii, 478.
- (13) HENDERSON: This Journal, 1909, xxiii, 354, fig. 3.
- (14) D. HEER: Arch. f. d. gesamt. Physiol., 1912, cxlviii, 1.
- (15) GARTEN: Zeitschr. f. Biol., 1915, lxvi, 52.
- (16) FRANK: Zeitschr. f. Biol., 1905, xlv, 495.

GASTRIC RESPONSE TO FOODS¹

XIII. THE INFLUENCE OF SUGARS AND CANDIES ON GASTRIC SECRETION

RAYMOND J. MILLER, OLAF BERGEIM, MARTIN E. REHFUSS
AND PHILIP B. HAWK

*From the Laboratory of Physiological Chemistry of Jefferson Medical College,
Philadelphia*

Received for publication May 21, 1920

The widespread use in the diet of large quantities of refined sugars and candies is a comparatively modern development. Because the races of men have lived for ages without general access to sugars in concentrated form, a question has naturally arisen as to whether the use of such foods may not in some instances give rise to harmful effects. It was perhaps natural also that the rapid development of the glucose industry should bring forth opponents and proponents of its wide use in cooking and in confections.

It is not necessary at this date to refute statements with reference to the alleged harmful character of cane sugar or glucose per se, inasmuch as it is well known that all digestible carbohydrates are absorbed from the intestine in the form of simple sugars and in the main as glucose formed from the starch of foods.

Certain objections to the use of sweets must, however, be considered. It cannot be denied that the eating of candies before meals decreases the appetite for other foods in general and that thus, particularly in the case of children, the intake of foods containing essential proteins, vitamins and inorganic salts may be reduced below the optimum requirements for growth. Purified sugars can obviously furnish but the single dietary essential, carbohydrate. This depression of appetite may be associated with the rapid absorption which sugars undergo in the intestine as well as with the depression of gastric secretion which is indicated by data presented in this paper. Such work as has been

¹ The expenses of this investigation were defrayed from funds furnished by Mrs. M. H. Henderson, The Curtis Publishing Company and Doctor L. M. Halsey.

carried out on the assimilation of carbohydrates as measured by the rise of blood and urinary sugar following their ingestion indicates that pure sugars are absorbed sooner from the intestine than the glucose formed from starch ingested (1). The assimilatory power of the normal human body for glucose would not appear, however, to be readily overtaxed.

It is known that in diabetes the weakened assimilatory function of the system for carbohydrate is still further impaired by the ingestion of large amounts of such food, and it might be supposed that habitual, long-continued use of many sweets might lead to the aggravation of a diabetic tendency which might not otherwise manifest itself. Such a suggestion has been made, but there is little concrete evidence to support it.

Sugars and candies, on the other hand, are particularly suited to furnish to the body, in convenient form, an additional quota of readily assimilable energy. Thus it has been pointed out that a single caramel may furnish 45 calories or sufficient energy for a mile walk (2), and that other confections yield similar amounts of energy. The view that these preparations are of negligible food value must, therefore, be discarded. Surely there is a physiological basis for the candy craving of children which cannot be disregarded, unless energy in abundance is furnished by other foods, nor does there seem to be any good reason for replacing more of the food carbohydrate by fats (except those high in vitamins) which are less readily assimilated and for which children generally have less desire.

In the cases of gastro-intestinal derangements more common in adult life, the influence of diet on the secretory, motor and fermentative processes of the digestive tract may overbalance considerations of energy value.

In the present paper we have endeavored to determine the influence of certain sugars, candies and other confections on the secretory and motor responses of the stomachs of normal adults.

The experiments were carried out on normal medical students and members of the staff of the department. They reported about nine o'clock in the morning, and any residuums which were present were removed from the stomach. The sugar solutions or candies were then given and samples of stomach contents removed at 15-minute intervals until the stomach was empty. Free and total acidities, pepsin, trypsin and amino acid nitrogen were determined by methods previously described (3).

The response of the stomach was studied following the ingestion of cream candies, hard candies, chewing candies, fresh and stale candies, chocolate and candy combinations. Inasmuch as most sweets enter the stomach essentially as sugar solutions, a preliminary study was made of the influence of concentrated and dilute solutions of cane sugar and glucose.

The response of the stomach to dilute and concentrated solutions of sucrose, glucose and maple sugar. Nine experiments were made on dilute and concentrated sugar solutions. The results of these experiments are charted in figures 1 to 9. One subject was given 250 cc. portions of 4 per cent glucose and cane sugar solutions; another subject, 150 cc. portions of 6 per cent glucose and cane sugar solutions, the total amount of sugar given in each case being about 10 grams. The same evacuation time (1 hour and 45 minutes) was obtained in each of the four experiments, and the acid responses were very similar.

No distinction could, therefore, be made between the responses of the stomach to dilute solutions of glucose and cane sugar nor, as the curves show, could there have been any distinct depression of gastric secretion by the dilute sugar solutions in the quantities given.

Maple sugar in dilute solution was given to the same subjects and was found to leave the stomach in from 45 minutes to an hour and 15 minutes without distinct depression of gastric secretion. The fact that this solution left sooner than the cane sugar or glucose may have had some relation to the more pleasant taste of the maple sugar. The cases are not quite comparable, however, as the glucose and cane sugar solutions were given without removing residuums.

Concentrated sugar solutions were given the men who had previously received dilute solutions. One subject was given 100 grams each of glucose and cane sugar in 59 per cent solutions. The other subject was given 100 grams of glucose in 40 per cent solution. Such solutions remained in the stomach from one-half to an hour longer than similar volumes of the dilute sugar solutions. In the case of the glucose solutions, the secretion of gastric acid was markedly depressed for an hour and a half or until much of the glucose had left the stomach. The secretion of pepsin was inhibited also. Cane sugar in concentrated solution appeared to have somewhat more stimulatory power, but its evacuation was likewise delayed. It is possible that the sweeter taste of cane sugar or its less rapid absorption from the intestine may influence the response of the stomach to its concentrated solution, but more evidence on this point would be required. It is clear that con-

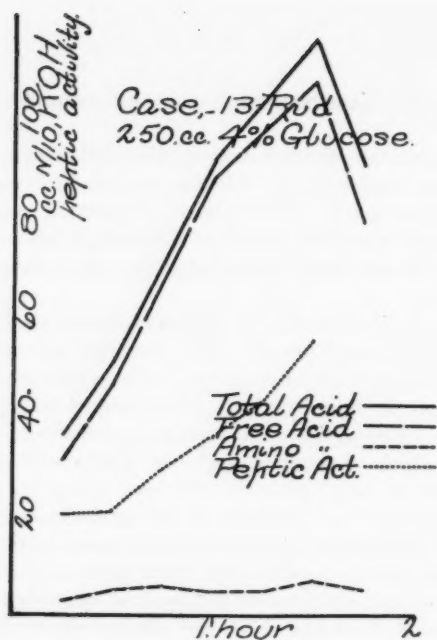


FIG. 1

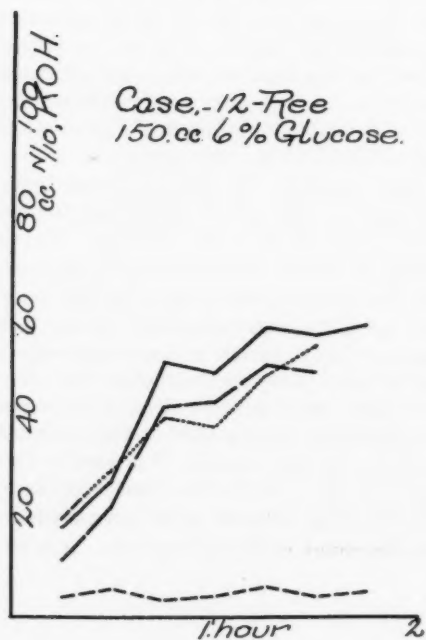


FIG. 2

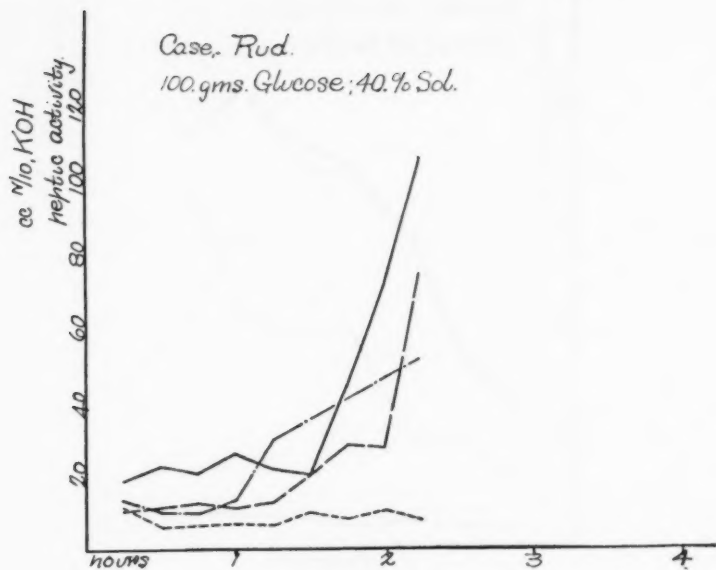


FIG. 3

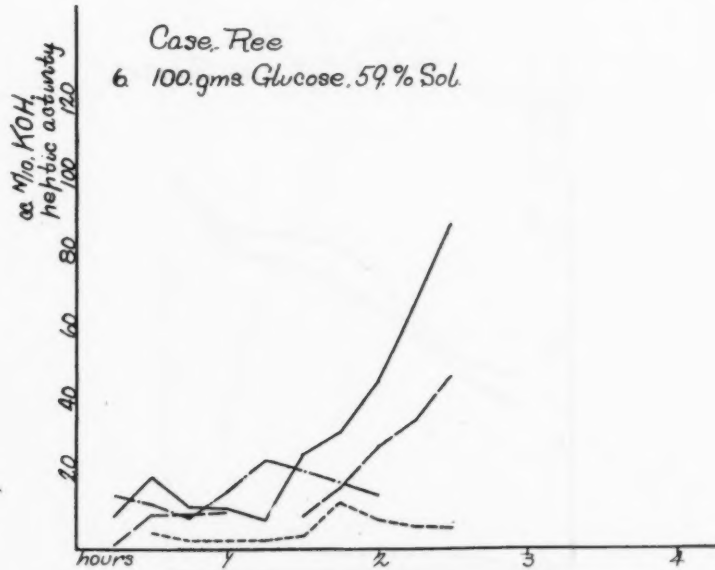


FIG. 4

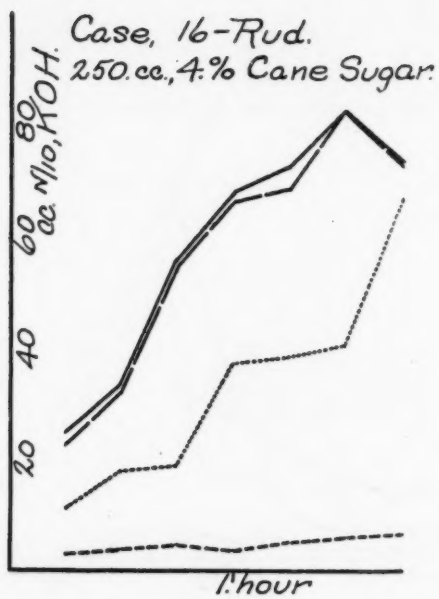


FIG. 5

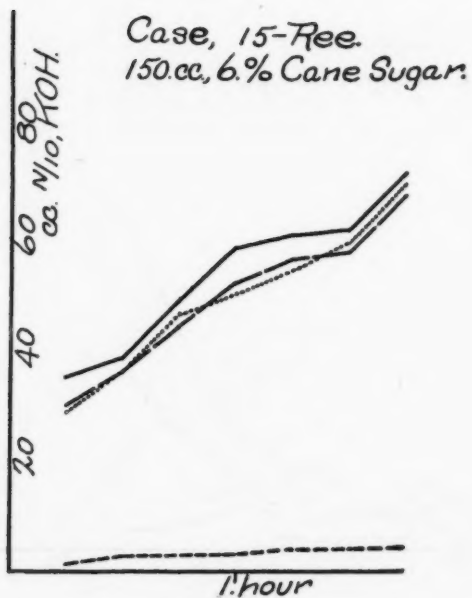


FIG. 6

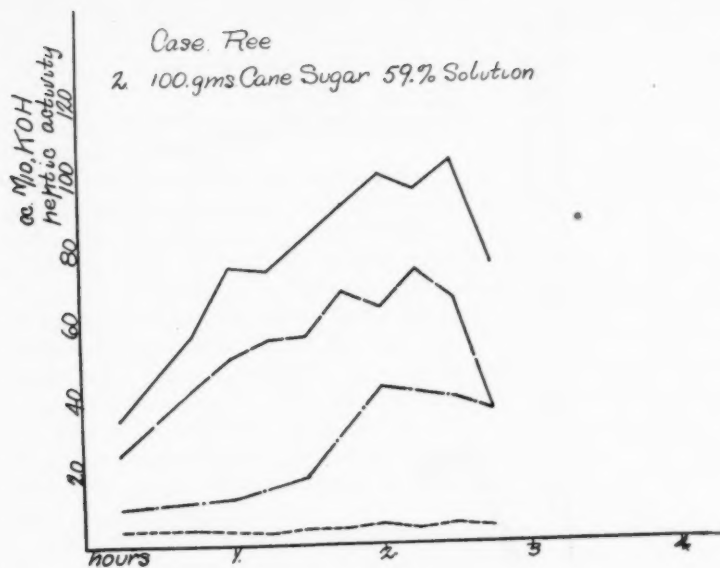


FIG. 7

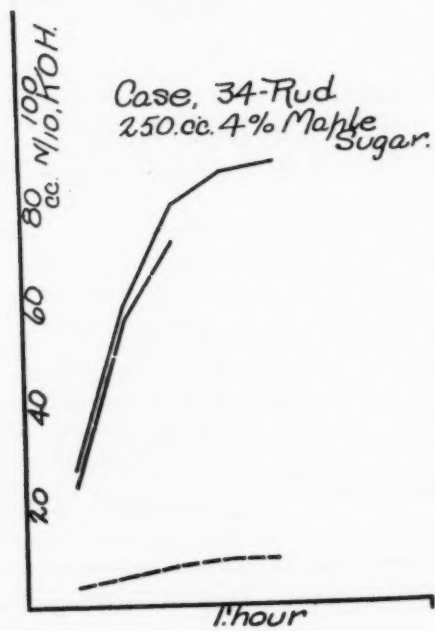


FIG. 8

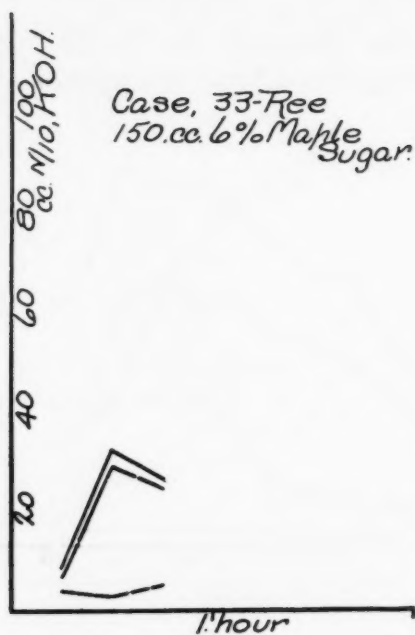


FIG. 9

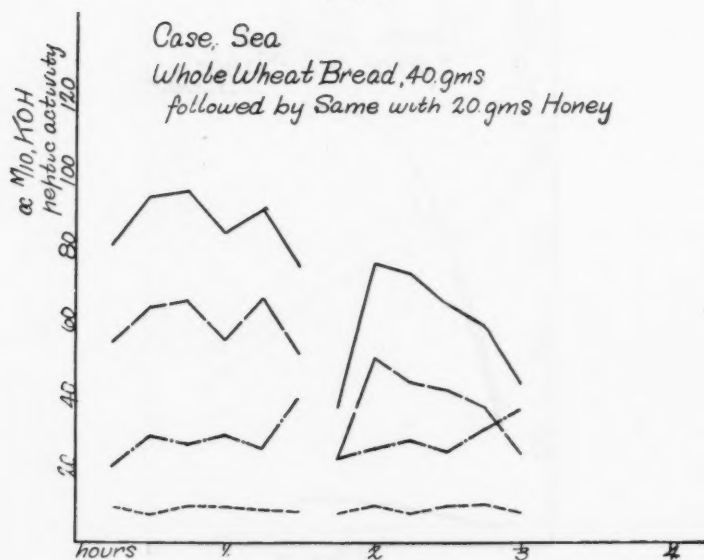


FIG. 10

centrated sugar solutions markedly depress gastric secretion and delay evacuation.

Soft candies. Under this heading were included chocolate creams, fudge, bonbons and wafers. The contents alone of chocolate creams, and plain milk chocolate were also studied.

The interiors of chocolate creams (consisting mainly of glucose) were given in 100-gram portions to two subjects (see figs. 11 and 12). It will be noticed that gastric secretion was markedly inhibited and evacuation much delayed by the ingestion of this amount of cream candy which was given without water and formed a concentrated sugar solution in the stomach. The same depressing action was noted where soft creamy bonbons were given (see fig. 13). These contained somewhat more cane sugar and were more highly flavored than the creams previously tested. This may possibly have accounted for the slightly more rapid evacuation.

Soft creamy wafers of strawberry flavor were given to one subject, 100 grams of the candy being ingested. As might be expected, the gastric secretion was depressed by the large amount of sugar present. Evacuation, however, was completed in moderate time ($1\frac{3}{4}$ hour), showing perhaps some influence of the fruit flavor on gastric motility.

Wafers of the same type but with strong peppermint flavor remained three-quarters of an hour longer in the stomach of this subject than did the strawberry wafers and gave rise to somewhat more acid secretion. The delayed evacuation may have been due to irritation of the duodenal mucosa by the oil of peppermint used as a flavoring agent.

Chocolate fudge remained in the stomach of one subject half an hour longer than a strawberry-flavored cream candy (see fig. 16). There was also a distinctly higher acid production in the case of fudge. Both of these effects must be related to the presence in the fudge of butter fat and chocolate.

That chocolate stimulates gastric secretion is indicated by our experiment in which milk chocolate was given (see fig. 17), an acidity twenty points higher being attained than in the case of creams. Milk constituents are probably responsible in part for this effect. Milk chocolate left the stomach in 2 hours or half an hour sooner than cream candy. It must, therefore, be considered as throwing less of a burden on the stomach than the latter.

Chocolate creams were compared in two cases with the contents of similar creams. Our best subject (see fig. 18) showed practically the same evacuation time for both but a somewhat higher acid develop-

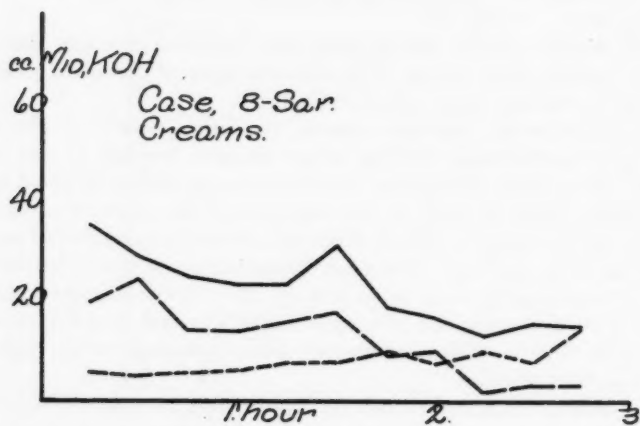


FIG. 11

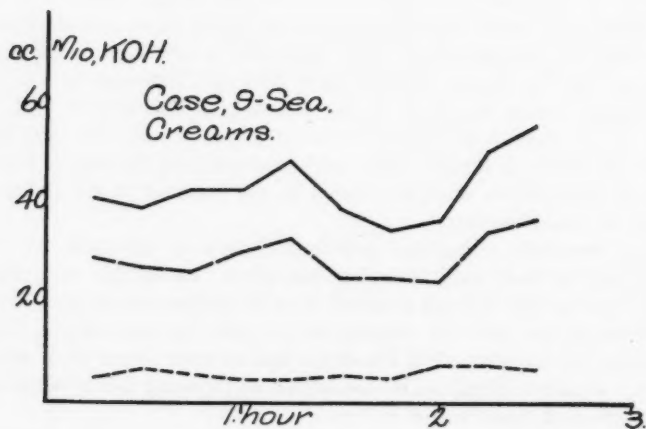


FIG. 12

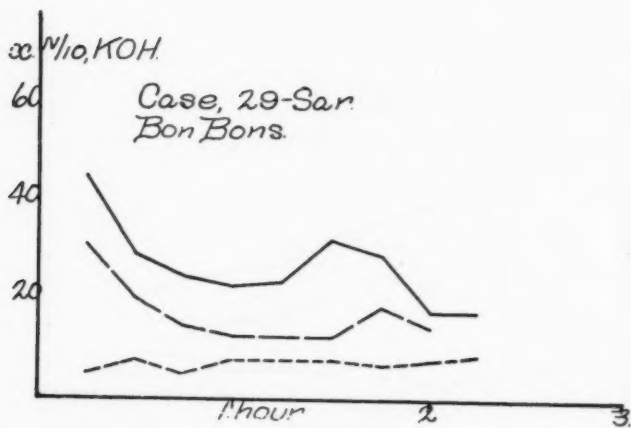


FIG. 13

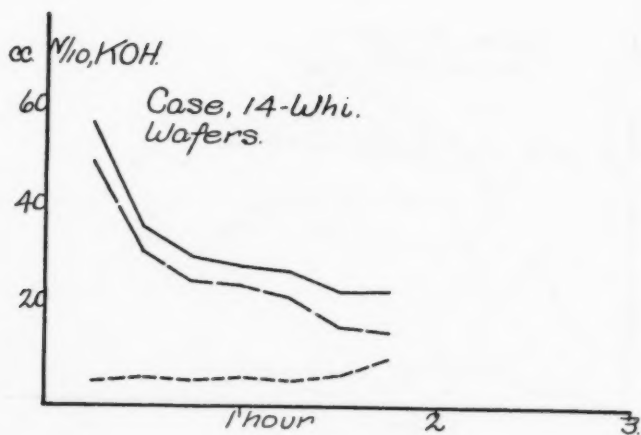


FIG. 14

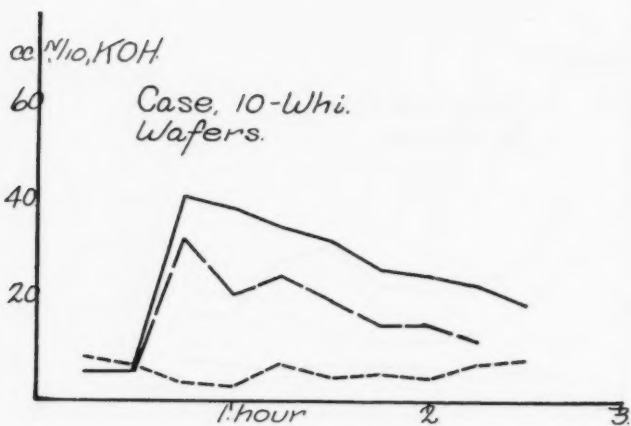


FIG. 15

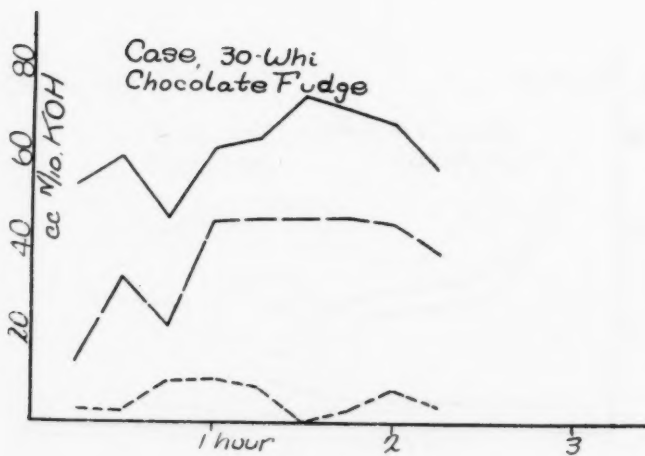


FIG. 16

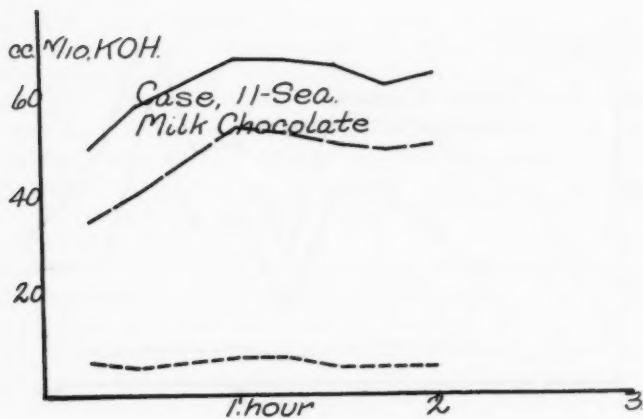


FIG. 17

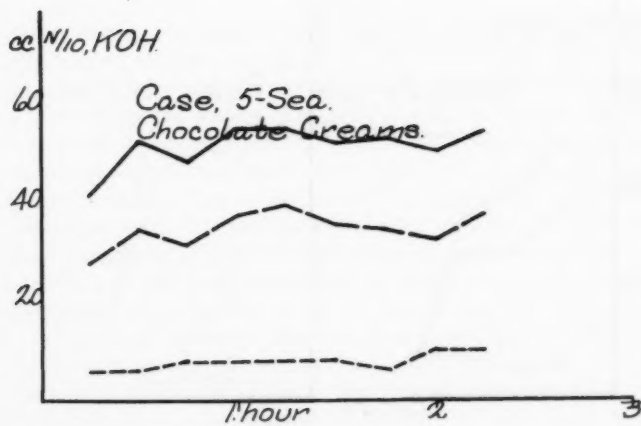


FIG. 18

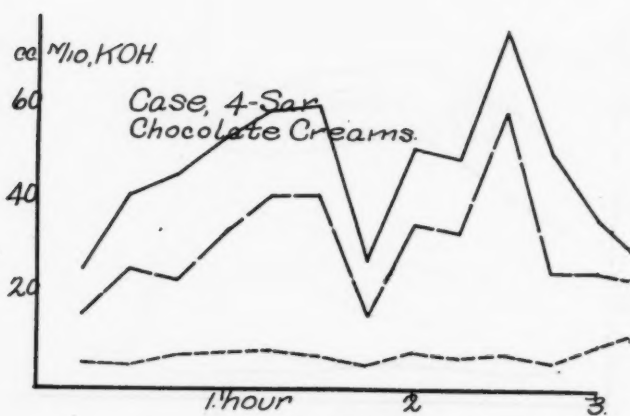


FIG. 19

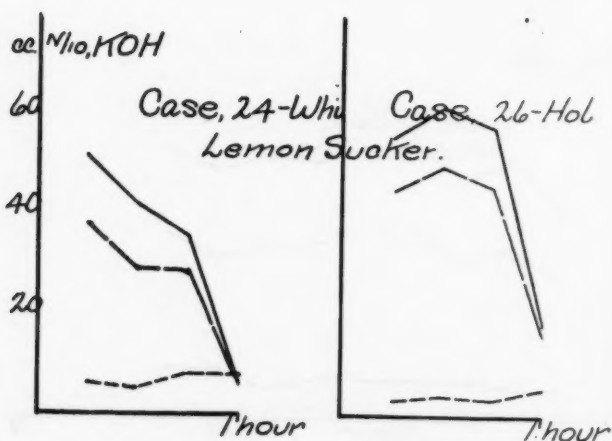


FIG. 20

FIG. 21

ment on chocolate creams in agreement with our findings on chocolate alone. Our second subject also gave higher acidities on chocolate creams but evacuation was delayed due to considerable intestinal regurgitation characteristic of this subject. He was also given, for comparison, chocolate creams which were 8 months old and very stale and not appetizing. These showed delayed evacuation and high acidity as compared with cream candies, and it appears probable were less easily handled by the stomach than similar candy in the fresh condition. Interpretation is somewhat difficult on account of the excessive regurgitation of this subject.

Hard candies. Two men were permitted to suck continuously for 15 minutes on hard candies (lemon-flavored stick candy). The candies were weighed before and after, and it was found that one man succeeded in obtaining 15 grams of candy, the other only .7 grams. No water was taken by either during the course of the experiment so that the sugar must have entered the stomach as a solution of moderate concentration. In each case a slight gastric secretion of moderate acidity was developed, and the stomach was empty in an hour. Neither was there any distinct continued secretion afterward. It is clear that the burden placed on the stomach by sucking the hard candy was very much less than that produced by the liberal or moderate eating of cream candies.

Chewing candies. Caramels, salt water taffy and gum drops were the chewing candies studied (see figs. 22 to 25). Caramels gave rise to a much greater acid production than cream candies, although evacuation times were about the same. This acidity may have been due to the greater chewing psychic secretion as well as to direct stimulation by ingredients of the caramels other than sugar. At any rate the marked depressing action of pure sugar candies was not noted. The marked differences between free and total acidities were due largely to the action of the gastric acid on the phosphates of swallowed saliva.

In the case of gum drops experimental difficulties were met with as the gelatinous mass formed in the stomach clogged the aspiration tube. It is clear, however, that the gum drops left the stomach in moderate time and produced little acid stimulation and were thus handled without difficulty so far as the stomach was concerned.

Salt water taffy left the stomach of one subject sooner than caramels or creams but developed a much lower acidity than caramels. It may be that the chewing psychic secretion caused by eating caramels was greater due to their flavor more nearly approximating that of palatable food normally giving rise to a gastric stimulation.

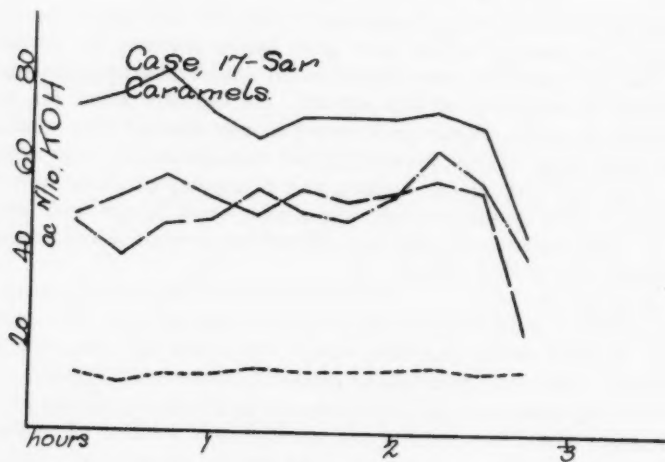


FIG. 22

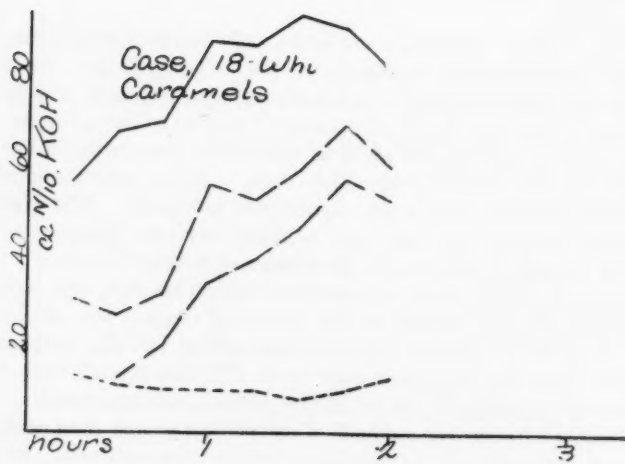


FIG. 23

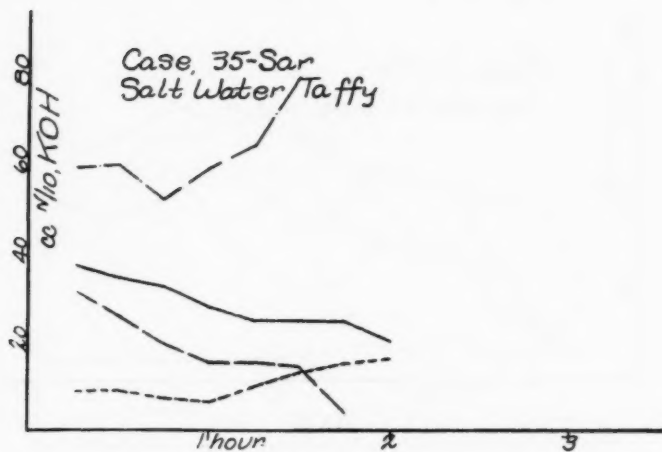


FIG. 24

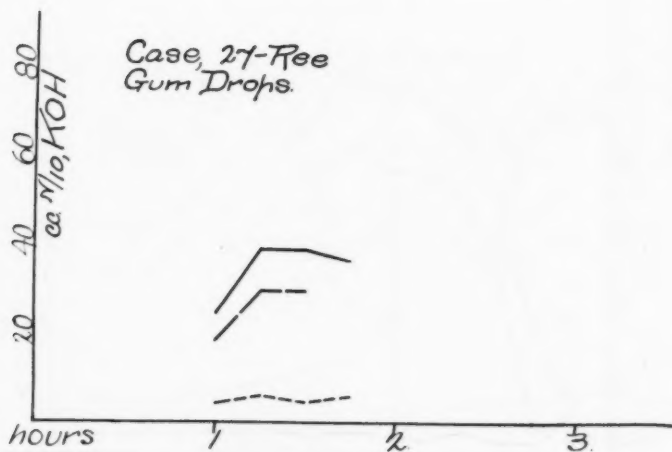


FIG. 25

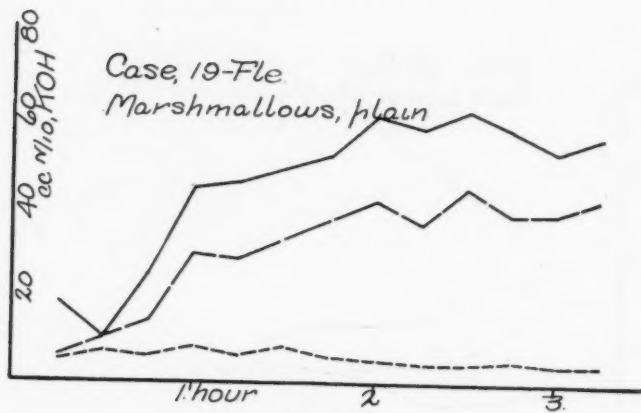


FIG. 26

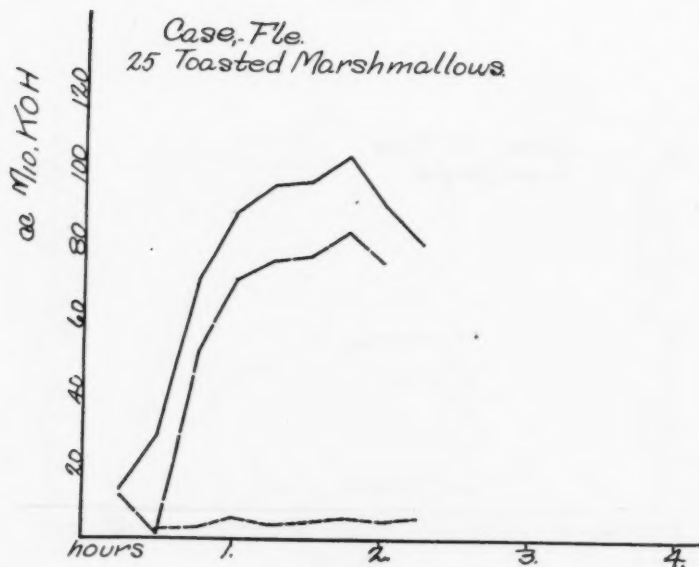


FIG. 27

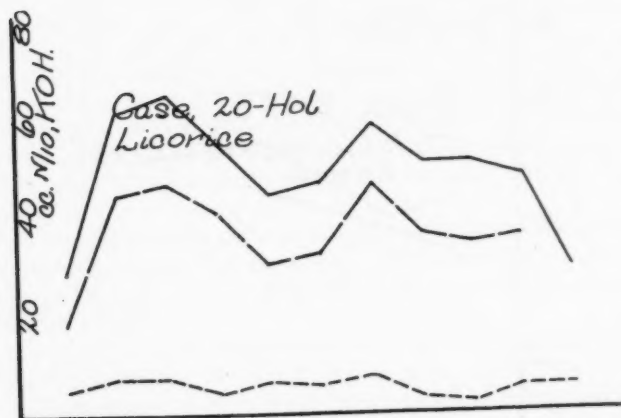


FIG. 28

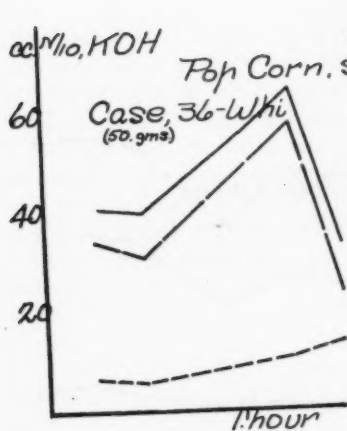


FIG. 29

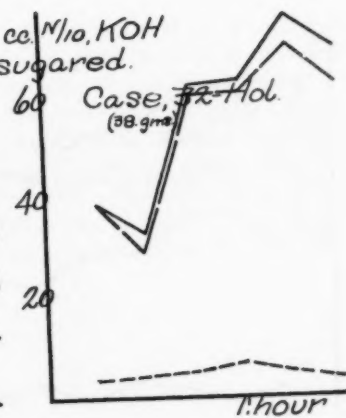


FIG. 30

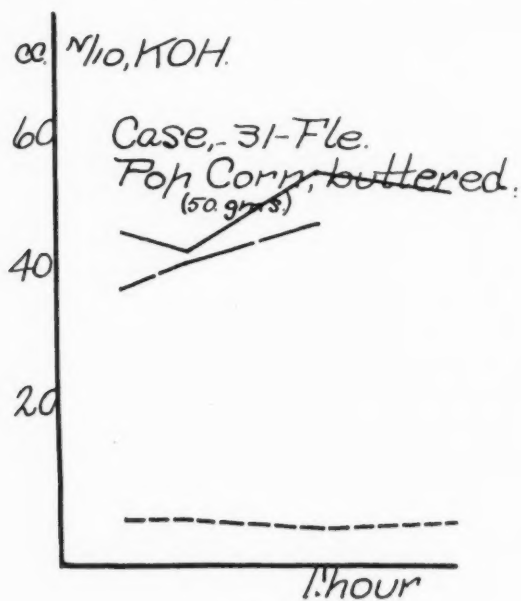


FIG. 31

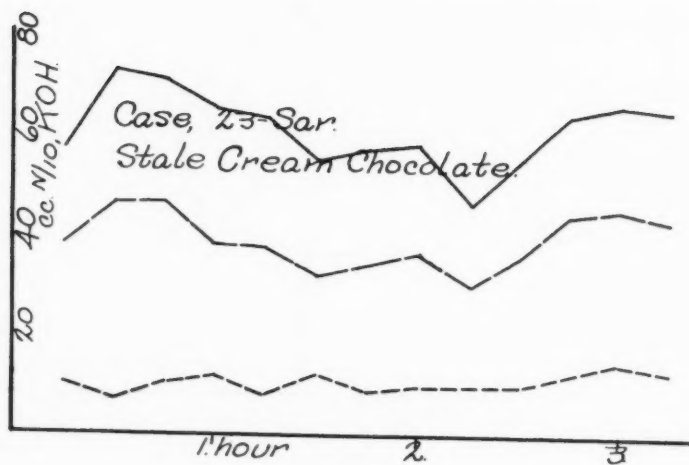


FIG. 32

Marshmallows and licorice. Toasted and untoasted marshmallows were given to one of our subjects. The gastric response was markedly different in the two cases. The toasted marshmallows left the stomach an hour sooner and gave rise to an acid development forty points higher than plain marshmallows. The psychic stimulation due to the more appetizing flavor after toasting may be partly responsible for such differences, as well as the alteration in texture and in the condition of the egg white which they contain. It is known that raw egg white has very little stimulatory power as compared with the cooked product.

A man was allowed to suck a stick of licorice for 15 minutes, obtaining in this time about 5 grams of the substance. It gave rise to a fairly abundant secretion of moderate acidity and remained in the stomach for $2\frac{3}{4}$ hours.

Pop-corn preparations. Sugared pop-corn was given to two subjects in amounts of 50 and 38 grams respectively. Such pop-corn was found to leave the stomach in about 1 hour and to develop a moderate acidity. Buttered pop-corn gave a very similar response in another subject (see fig. 31), leaving for the most part in an hour and a half. It must be borne in mind, however, that certain of the larger and harder particles of corn which could not be aspirated remained in the stomach somewhat longer. Practically all of the corn left in moderate time.

Bread and honey. Sugars being very frequently given in the form of syrups added to bread and other foods, we endeavored to determine what effect such additions might have upon the gastric response.

A man was given 40 grams of whole wheat bread without additions, and after this had left the stomach he was given the same amount of bread with 20 grams of honey. The addition of honey depressed gastric secretion slightly but did not delay evacuation, although the food value of the preparation had been greatly increased. A moderate amount of honey added to bread cannot, therefore, be considered harmful.

TABLE I
The response of the human stomach to candies

SUBJECT NUMBER	PREPARATION	EVACUA- TION TIME	HIGHEST TOTAL ACIDITY	TOTAL ACIDITY AT ONE HOUR
1. Rud	Dilute glucose solution.....	1:45	107.5	90.0
2. Ree	Dilute glucose solution.....	1:45	57.5	48.0
3. Rud	Concentrated glucose solution.....	2:15	106.0	28.0
4. Ree	Concentrated glucose solution.....	2:30	86.5	10.0
5. Rud	Dilute cane sugar solution.....	1:45	82.0	68.0
6. Ree	Dilute cane sugar solution.....	1:45	71.0	58.0
7. Ree	Concentrated cane sugar solution.....	2:45	99.5	95.0
8. Rud	Dilute maple sugar solution.....	1:15	88.5	86.0
9. Ree	Dilute maple sugar solution.....	0:45	32.5	32.5
10. Sea	Chocolate creams.....	2:15	56.5	56.0
11. Sar	Chocolate creams.....	3:15	79.5	54.0
12. Sar	Creams.....	2:45	36.0	24.0
13. Sea	Creams.....	2:30	57.5	44.0
14. Whi	Chocolate fudge.....	2:15	73.0	61.0
15. Sea	Milk chocolate.....	2:00	69.5	69.0
16. Sar	Bonbons.....	2:30	33.0	24.0
17. Whi	Cream wafers, peppermint.....	2:30	42.5	40.0
18. Whi	Cream wafers, strawberry.....	1:45	37.5	29.0
19. Whi	Lemon sticks.....	1:00	52.0	6.0
20. Hol	Lemon sticks.....	1:00	62.0	18.0
21. Sar	Caramels.....	2:45	80.0	71.0
22. Whi	Caramels.....	2:45	92.0	86.0
23. Sar	Taffy, salt water.....	1:45	26.5	26.0
24. Hol	Licorice.....	2:45	64.0	54.0
25. Ree	Gum drops.....	1:30	38.5	24.0
26. Fle	Marshmallows, plain.....	3:15	61.5	43.0
27. Fle	Marshmallows, toasted.....	2:15	103.0	88.0
28. Sea	Bread and honey.....	1:30	75.0	64.0
29. Whi	Pop-corn, sugared.....	1:30	65.5	58.0
30. Hol	Pop-corn, sugared.....	1:30	78.5	64.0
31. Fle	Pop-corn, buttered.....	1:30	56.5	56.0
32. Sar	Stale chocolate creams.....	3:15	72.5	64.0

SUMMARY AND CONCLUSIONS

Large amounts (100 grams) of cane sugar or glucose in concentrated solution markedly depressed gastric secretion and delayed evacuation of the stomach.

Small amounts (10 grams) of cane sugar or glucose did not appreciably inhibit either gastric secretion or evacuation.

Candies depress secretion and delay evacuation in proportion to their sugar content and the amounts of them ingested. This tendency is influenced, however, by flavoring substances, and particularly by added food ingredients such as milk, eggs or chocolate, which stimulate gastric secretion.

Candies should be eaten not before but after meals. Hard candies which must be sucked are preferable to cream candies for children because of the smaller quantity of less concentrated sugar solution derived from them.

Cane sugar and maple sugar elicited much the same response from the human stomach as glucose, although the possibility that the greater sweetness and less rapid absorption of the first mentioned sugars gives them a slight advantage is not excluded.

Soft candies such as bonbons, soft creamy wafers and the interiors of chocolate creams when given in 100-gram portions exerted the same depressing action on gastric secretion and evacuation as concentrated sugar solutions.

Peppermint oil used as a flavoring agent delayed evacuation while a strawberry fruit flavor appeared to accelerate it.

Chocolate appeared to stimulate gastric secretion as indicated by experiments on milk chocolate, chocolate fudge and chocolate creams, which gave higher acid figures than plain sugar candies. Stale chocolates remained in the stomach relatively long.

The sucking of hard candies introduced but a small amount of sugar into the stomach which was readily evacuated and exerted little depressing action on gastric secretion.

Chewing caramels gave rise to a more voluminous gastric secretion than cream candies, but evacuation times were about the same. Salt water taffy gave rise to less secretion, while gum drops left the stomach rapidly with little acid production.

Plain marshmallows remained in the stomach rather long, but after being toasted these confections left the stomach rapidly and gave rise to high intragastric acidities.

Licorice gave rise to a fairly abundant secretion and remained in the stomach for nearly 3 hours.

Sugared or buttered pop-corn developed a moderate acidity and left the stomach rather quickly.

The addition of honey to bread did not delay evacuation, although acid production was somewhat depressed.

BIBLIOGRAPHY

- (1) JACOBSON: Biochem. Zeitschr., 1913, lvi, 471.
- (2) BENEDICT AND BENEDICT: Boston Med. Surg. Journ., 1918, clxxix, 153; 1919, clxxxi, 415.
- (3) SMITH, FISHBACK, BERGEIM, LICHTENTHAELER, REHFUSS AND HAWK: This Journal, 1919, xlix, 174.

FURTHER EVIDENCE ON THE FUNCTIONAL CORRELATION OF THE HYPOPHYSIS AND THE THYROID

JOHN A. LARSON

*From the Rudolph Sprechels Physiological Laboratory of the University of
California*

Received for publication May 26, 1920

In a former paper (1) I presented evidence to show that the administration of the anterior lobe of the pituitary has a very beneficial action upon maintenance and growth of thyroidectomized rats. In that paper I pointed out the importance of using animals of "approximately the same size, age and strain, and wherever possible from the same litter." At that time however, I was unable to meet these conditions in a wholly satisfactory way. I have now repeated the experiments with a larger number of animals and with strict regard to the comparison of individuals from a single litter.

There were in all seventy-two litters used. These were selected of as nearly the same date of birth as the conditions of the work would permit. The litters were kept separated and directly upon the day of weaning were ear-marked and subdivided into the respective series. In this arrangement extreme care was taken in regard to sex, weight and even color. All runts, individuals below the average weight and size of the litter, were eliminated. In anticipation of possible accidental deaths from ether, etc., the members in the operated series greatly outnumbered those in the normal series. There were four main series of animals:

A. Thyroidectomized animals fed upon the normal diet in addition to kidney.

B. Thyroidectomized animals fed upon the normal diet in addition to anterior lobe.

C. Normal, that is, unoperated, animals fed upon the normal diet in addition to anterior lobe.

D. Normal, that is, unoperated, animals fed upon the normal diet in addition to kidney.

To these series a fifth, E, was added, consisting of only a very few thyroidectomized animals which were fed upon the normal diet in addition to thyroid. As was to be expected from the results of thyroid feeding in cases of thyroid deficiency in other animals and also from the work of Cramer and McCall (2) on the rat, the animals in group E differed scarcely at all from the normals. The individual results will not be given in the tables, but the final results will be recapitulated with the other four series.

In the subdivision of the litter one rat of the same sex and color was placed in each of the four main series. This was possible in most cases, since the number in the litters varied from four to twelve. But if there happened to be, say, two males and two females in one litter, those of the same sex were placed in the operated series and the others in the unoperated. After picking out one animal for each series all of the remaining litter mates were distributed strictly according to sex in the operated groups, there being sometimes four from one litter in each operated series. Any odd member was placed in the fifth group. The result was that at the end of the division every member in each of the four series had a litter mate of the same sex in every other series. I have thus been able to give tables in which it is possible to see the results in each single litter although as a matter of fact the whole number of animals used was so large that a statistical treatment would have served the same purpose.

So far as possible, litter mates were operated upon on the same day.

The experiment lasted from August 1, 1919, the date of the first operation, until March 1, 1920, the time of the last weighing. The animals were weighed separately and at the same time, in relation to feeding, once a week until through December and then every two weeks until January 19, and the final weighings were made at the end of the experiment on March 1.

The rats were kept five in a cage. The cages contained only males or females in order to avoid distortion of growth curve through pregnancy. The food which I have spoken of above as "normal diet" consisted of the following ingredients:

Corn meal.....	6.0 parts
Rice.....	2.0 parts
Barley.....	2.0 parts
Meat powder.....	4.5 parts
Lard.....	5.5 parts
NaCl.....	1.0 per cent
CaCl ₂	1.5 per cent
Greens ad libitum	

In addition to the normal diet each animal received as already stated either anterior lobe of the pituitary or an equivalent amount of kidney. The members of group E received 0.2 gram of fresh beef thyroid daily; whenever possible the dosage of anterior lobe was one entire lobe daily to each animal in the two series, and whenever there were too few glands for that dosage they were divided equally between the different members. The anterior lobes were administered in such a way that each animal received at least one-half a gland at each feeding.

The histories of all the members of the series are recorded in two main tables. Throughout the following discussion no reference is made to animals which were killed by ether or in a few cases, lost. Such individuals were discarded.

In the first table the histories of all of the animals still living at the termination of the experiment are exhibited apart from those rats which died. These latter are treated in tables 3 and 4.

Table 1 shows the sex, number, date of birth, date of operation, initial and terminal weights as well as the gain. Up to no. 65, the number, sex and date of birth are the same for all of the individuals represented in a horizontal row, since all of the animals therein are members of the same litter. In litters 65, 66 and 67, the members are of the same sex in the operated group but of a different sex from that in the normal groups, which is the same, however, in the two unoperated series. The members of groups 73 to 81 are from different litters owing to deaths of the other litter mates.

Table 2 presents the averages of the data shown in detail in table 1. The homogeneity at the outset of the experiment is indicated by the average initial weights which are 32.9, 32.5, 31.8 and 31.8 respectively. In contrast with these the average terminal weights (and the average gains) show marked differences. Growth was slower in the thyroidectomized animals fed on the control diet than in the thyroidectomized animals which received pituitary, the gain of the former being only 123.7 grams as compared with 171.3 grams. This result is in conformity with that contained in the previous paper. It must be remembered, moreover, that these figures deal only with the animals which survived at the close of the experiment. If in calculating the effect upon the rate of growth the animals which did not survive to the end had been included, the difference would have been still more marked.

It is also of interest to note that in the normal series the gain in weight was greater in the pituitary animals than in those receiving the control diet. Under the circumstances there can scarcely be a

TABLE 1

NUMBER OF LITTER	SEX	SERIES A				SERIES B				SERIES C				SERIES D				SERIES E			
		Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	
1	♂	6/22	8/14	38	204	166	8/13	39	238	199	36	262	226	37	206	169	35	218	183		
2	♂	6/23	8/7	59	182	123	8/4	51	268	217	45	296	251	54	242	188	32	192	160		
3	♀	6/24	8/12	21	136	115	8/12	27	195	168	25	174	149	26	182	156					
4	♂	6/24	8/13	26	100	74	8/12	25	190	165	30	230	200	28	224	196					
5	♀	6/24	8/14	41	130	89	8/13	35	176	141	25	186	161	26	174	148					
6	♀	6/25	8/11	41	174	133	8/11	37	170	133	36	186	150	35	150	115					
7	♀	6/25	8/15	26	126	100	8/15	38	198	160	31	220	199	25	202	177					
8	♀	6/26	8/11	39	126	87	8/11	35	195	160	36	180	144	33	170	137	24	144	120		
9	♀	6/26	8/11	43	98	55	8/11	46	160	114	50	210	160	26	182	156	42	162	130		
10	♀	6/26	8/12	28	166	138	8/12	34	240	206	33	170	137	35	150	115					
11	♀	6/26	8/14	33	144	111	8/13	29	190	161	33	204	171	28	150	122					
12	♀	6/27	8/15	30	122	92	8/15	36	195	159	32	154	122	35	112	77					
13	♀	6/27	8/15	30	174	144	8/15	36	220	184	42	142	100	38	172	134					
14	♂	6/27	8/14	29	198	169	8/13	35	230	215	34	264	230	34	228	194					
15	♂	7/3	8/22	36	210	180	8/20	35	260	225	51	272	221	47	204	157	47	248	201		
16	♂	7/8	8/26	35	216	171	8/22	32	235	203	37	214	177	41	194	153	31	234	200		
17	♂	7/9	8/27	38	192	154	8/27	26	208	182	34	302	268	35	258	223					
18	♀	7/9	8/29	35	164	129	8/24	33	160	127	36	204	168	25	168	143					
19	♀	7/10	8/19	33	112	79	8/18	39	168	129	26	136	110	23	144	121					
20	♀	7/10	8/22	41	186	145	8/20	30	232	202	28	191	166	24	176	152					
21	♀	7/10	8/22	28	174	146	8/20	30	196	166	32	168	136	30	124	99					
22	♂	7/10	8/22	29	174	145	8/21	39	266	227	33	264	231	37	220	183					
23	♀	7/12	8/14	32	180	148	8/14	31	232	201	35	130	95	36	170	134					
24	♀	7/13	8/26	36	178	142	8/29	33	208	175	27	180	153	27	180	153					
25	♂	7/14	9/1	22	304	282	8/29	30	202	272	20	304	284	21	308	287					

26	♀	7/26	9/4	29	136	107	9/3	26	206	180	33	174	141	35	156	121	27	180	153
27	♀	7/26	9/5	35	156	121	9/5	35	180	145	43	184	141	44	172	128			
28	♀	7/26	9/6	41	166	125	9/5	35	195	160	47	210	163	48	196	148			
29	♂	8/8	9/24	33	188	155	9/24	20	235	205	21	234	213	22	206	184	46	226	180
30	♀	8/8	9/26	22	134	112	9/26	18	195	177	28	176	148	27	190	163	32	178	146
31	♀	8/8	9/26	25	134	109	9/26	24	195	171	26	154	128	26	172	136			
32	♀	8/10	10/12	27	150	123	10/12	26	230	201	29	160	131	31	138	107			
33	♀	8/10	10/12	25	114	89	10/12	24	175	151	30	150	120	31	144	113	30	138	108
34	♀	8/11	10/6	26	138	112	10/6	35	170	135	28	166	138	28	164	136			
35	♀	8/11	10/2	34	136	102	10/2	33	190	157	19	160	141	19	134	115	31	154	123
36	♂	8/11	9/22	35	214	179	9/22	38	230	192	33	286	233	33	236	203	31	246	215
37	♀	8/12	9/25	34	164	130	9/24	21	230	209	31	188	157	33	178	145			
38	♀	8/12	10/15	34	132	98	10/7	34	160	156	31	180	149	34	196	162			
39	♀	8/13	10/15	33	152	119	10/7	31	170	139	32	170	138	32	164	132			
40	♂	8/13	9/25	33	236	203	9/24	30	204	174	27	250	223	27	250	223			
41	♀	8/13	10/22	30	140	110	10/23	30	224	194	31	188	157	29	182	153	31		
42	♂	8/13	9/22	52	174	122	9/22	46	170	121	30	232	202	28	170	142			
43	♀	8/13	10/20	22	162	140	10/20	40	192	152	28	204	176	34	166	132			
44	♀	8/13	10/16	44	140	96	10/20	27	195	168	30	210	180	33	158	125			
45	♂	8/14	9/23	38	214	176	9/23	24	250	226	35	242	207	35	204	169			
46	♂	8/14	10/19	26	136	110	10/23	27	210	183	31	240	209	29	226	197			
47	♂	8/14	9/22	39	84	45	9/22	26	240	214	22	236	214	21	180	159			
48	♀	8/14	10/15	30	160	130	10/9	30	175	145	37	180	143	34	152	118			
49	♀	8/14	10/6	26	80	54	10/7	28	180	152	32	172	140	34	122	88			
50	♂	8/16	9/25	28	162	134	9/24	24	240	216	50	292	242	35	216	181			
51	♀	8/18	9/24	21	108	87	9/26	20	195	175	41	200	159	44	232	188			
52	♀	8/18	9/30	21	170	149	10/1	29	246	217	21	222	201	22	232	210			
53	♀	8/19	10/15	24	138	114	10/15	24	190	166	37	132	95	39	130	91			
54	♂	8/19	10/23	26	164	188	10/22	37	185	148	41	256	215	35	244	209			
55	♂	6/28	8/14	45	148	103	8/16	72	235	203	36	239	203	38	206	188			
56	♂	6/27	8/14	43	214	171	8/16	72	235	163	34	212	178	34	228	194			
57	♂	6/22	8/1	35	100	65	8/15	34	130	96	23	278	235	28	270	212			

TABLE 1—Continued

NUMBER OF LITTER	SEX	SERIES A				SERIES B				SERIES C			SERIES D			SERIES E				
		Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Initial weight	Terminal weight	Gain	
				grams	grams	grams		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	
58	♂	6/22	8/7	59	182	123		8/13	25	230	205	31	280	249	31	216	185			
59	♂	7/11	9/1	23	54	31		8/29	26	220	194	35	288	253	35	242	201			
60	♂	8/8	9/24	22	120	98		9/24	24	240	216	36	230	194	34	230	196			
61	♂	8/16	9/30	34	162	128		9/22	38	220	192	43	286	243	42	240	198			
62	♂	7/15	8/27	32	202	170		9/5	45	260	215	33	286	253	39	260	221			
63	♀	8/19	10/23	32	148	116		10/22	31	180	149	23	200	177	28	206	178			
64	♀	8/19	10/26	28	140	112		10/26	28	230	202	35	278	243	36	228	192			
65	♀	7/11	9/1	31	182	151		9/23	33	180	149	35	266	231	30	226	196			
66	♀	7/15	9/1	36	164	128		9/22	21	149	128	26	204	175	30	226	196			
67	♀	8/28	9/25	28	184	156		9/7	31	230	199	35	266	231	30	226	196			
68	♀	7/17	9/16	47	150	103		9/16	31	180	149	35	266	231	30	226	196			
69	♀	7/13	8/29	27	170	143		8/29	29	160	131	35	266	231	30	226	196			
70	♀	6/25	8/14	32	152	120		8/13	34	168	134	35	266	231	30	226	196			
71	♀	6/23	8/7	59	160	101		8/14	25	200	175	35	266	231	30	226	196			
72	♀	6/24	8/13	23	192	169		6/26	8/19	33	160	127	35	266	231	30	226	196		
73	♀	7/23	9/3	40	146	106		6/26	9/5	25	96	71	35	266	231	30	226	196		
74	♂	6/24	8/15	27	154	127		6/23	8/12	31	130	99	35	266	231	30	226	196		
75	♀	8/19	10/26	27	134	107		8/16	8/13	42	146	104	35	266	231	30	226	196		
76	♀	7/14	8/13	34	146	112		7/12	8/29	49	244	195	35	266	231	30	226	196		
77	♂	6/23	8/14	47	90	43		6/26	8/12	38	100	62	35	266	231	30	226	196		
78	♂	6/24	8/15	32	152	120		6/26	8/13	25	176	149	35	266	231	30	226	196		
79	♀	7/11	9/1	30	100	70		7/15	8/29	27	182	155	35	266	231	30	226	196		
80	♀	6/24	8/11	31	158	127		6/26	8/5	37	200	223	35	266	231	30	226	196		
81								6/24	8/6	56	280	224	35	266	231	30	226	196		
Averages.....				32.9	156.6	123.7			32.5	203	847.3	31	81	212.7	180.9	31.8	193.5	161.7		

TABLE 2

Summary of results shown in table 1

	NUMBER IN GROUP	AVERAGE INITIAL WEIGHT	AVERAGE TERMINAL WEIGHT	AVERAGE GAIN
		grams	grams	grams
Series A.....	80	32.9	156.6	123.7
Series B.....	81	32.5	203.8	171.3
Series C.....	67	31.8	212.7	180.9
Series D.....	67	31.8	193.5	161.7
Series E.....	12	33.7	193.3	159.6

TABLE 3A

Average gain for each sex

	A. THYROIDECTOMIZED + KIDNEY			B. THYROIDECTOMIZED + ANTERIOR LOBE			C. NORMAL + ANTERIOR LOBE			D. NORMAL + KIDNEY		
	Average initial weight	Average terminal weight	Average gain	Average initial weight	Average terminal weight	Average gain	Average initial weight	Average terminal weight	Average gain	Average initial weight	Average terminal weight	Average gain
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
Males.....	34.4	173.8	139.4	34	206.8	172.8	33.3	252.7	219.4	33.1	225.9	192.8
Females....	31.3	158.6	127.3	32	184.1	152.1	32.4	182.3	149.9	31.3	165.4	144.1

TABLE 3B

Total percentage gains; comparisons are made between members of the same sex under different conditions

	A. THYROID + KIDNEY	B. THYROID + ANTERIOR LOBE	DIFFERENCE BETWEEN A AND B	C. NORMAL + ANTERIOR LOBE	D. NORMAL + KIDNEY	DIFFERENCE BETWEEN C AND D
	per cent	per cent	per cent	per cent	per cent	per cent
Males.....	405.2	508.2	103.0	658.8	582.4	76.4
Females.....	306.2	406.7	100.0	462.6	425.7	33.9

TABLE 3C

Percentage gains of males over females; comparisons are made between the males and females receiving the same treatment. The figures here recorded represent the gains of the males over the females

A. THYROID + KIDNEY	B. THYROID + ANTERIOR LOBE	C. NORMAL + ANTERIOR LOBE	D. NORMAL + KIDNEY
per cent	per cent	per cent	per cent
9.5	13.6	46.3	36.3

TABLE 4

Animals dying during experiment, showing days of life after operation

NUMBER OF LITTER	SEX	DAYS OF LIFE	NUMBER OF LITTER	SEX	DAYS OF LIFE
Series A					
1	♂	204	36	♂	61
2	♂	33	38	♀	27
3	♀	201	42	♂	117
4	♂	152	43	♀	118
5	♀	189	44	♀	47
6	♀	37	45	♂	31
7	♀	44	48	♀	169
8	♀	46	49	♂	78
9	♀	24	51	♀	55
10	♀	168	52	♂	201
11	♀	192	53	♀	32
12	♀	133	54	♂	198
13	♀	24	55	♂	198
14	♂	24	56	♂	1
15	♂	196	57	♂	31
16	♂	38	58	♂	55
17	♂	44	59	♂	171
18	♀	23	60	♂	2
19	♀	76	61	♂	34
20	♀	131	62	♂	1
21	♀	23	63	♀	3
22	♂	166	65	♀	191
23	♀	195	66	♀	4
24	♀	168	67	♀	22
25	♂	127	70	♀	8
26	♀	24	71	♀	13
27	♀	31	72	♀	217
28	♀	182	4A	♂	93
31	♀	61	5A	♀	162
34	♀	71	56 A	♂	93
Series B					
3	♀	38	29	♂	31
10	♀	8	31	♀	34
15	♂	65	34	♀	32
21	♀	27	36	♂	41
22	♂	11	39	♀	127

doubt that the difference is due to a stimulating influence of the anterior lobe.

It is conceivable that the results might be different for the two sexes. I have tabulated the gains for the males and females separately in table 3. Here again the anterior lobe exerts a beneficial action upon the operated animals. In addition a comparison of the two sexes of the normal animal seems to reveal an increased effect of the anterior lobe upon the growth of males.

As stated above, tables 1 and 2 include only animals which were alive at the end of the experiment and are therefore useful in showing the influence of the gland substance on the rate of growth. Another important feature of the effect of the gland substance is seen in its influence upon the duration of life. Table 4 gives the length of life after the operation up to death and table 5 shows the total number of animals in each series as well as the number which died and the percentage of deaths.

TABLE 5
Tabular summary of deaths

	IN SERIES A	IN SERIES B	IN SERIES C	IN SERIES D	IN SERIES E
Total number.....	140	91	67	67	12
Number deaths.....	60	10	00	00	00
Number alive.....	80	81	67	67	12
Per cent deaths.....	42.8	10.9	0	0	0

DISCUSSION OF RESULTS

The experimental results show definitely that the administration of the anterior lobe of the hypophysis increases the growth and prolongs the life of thyroidectomized rats. Also the pituitary substance accelerates the growth of normal animals. These results at once suggest two possible interpretations: either *a*, that this beneficial effect is due to the direct substitution of the pituitary substance for that of the thyroid; or *b*, to a favorable action of the hypophysis upon the organism as a whole. The latter alternative is supported by the increased growth of the normal animals to which pituitary had been administered. On the other hand the difference in the two groups of operated animals is so much greater than in the two groups of normal animals that it hardly seems possible that this variance can be accounted for by

a general improvement of the state of the animals but rather that it is due in part, at least, to an actual substitution of the hypophyseal substance for the lacking thyroid secretion. This possibility is rendered more probable by the effect of the pituitary on the prolongation of life of the operated animals. For a further discussion of these possibilities and the literature, reference is made to the previous paper (1).

Since the publication of the previous paper three investigations have appeared which have an interesting bearing. All three deal with the effect of the administration of pituitary to thyroidectomized frog larvae. Smith and Allen (4) working independently report negative results. Hoskins and Hoskins (3) on the other hand, not only obtained beneficial effects but found that pituitary causes a hastened metamorphosis. They interpret these results as indicating either a substitution or an effect of the pituitary upon the whole organism. Thus the results in the previous work, the first positive indication of the possibility of a substitution, have been confirmed by the author and by Hoskins on different animals.

The question now arises as to the cause of death of the operated animals. Post mortems were made of all the animals and in not a single case was there any macroscopic evidence of infection in the operated region. Invariably the animal was extremely emaciated, undersized and with scanty hair. The cause of death may be fairly attributed to metabolic disturbances and interference with the normal bodily functions due to lack of thyroid secretion.

Careful pathological examinations of three animals were made by three different pathologists. In one, no pathological condition save that of extreme emaciation could be detected, while in another evidences of a pneumonia were present. In the last one examined but little of significance was found, the pathologist agreeing with me that even if the animal had died of some terminal disease such as pneumonia the "evidence is sufficient to make a diagnosis of terminal pneumonia developing in an animal whose resistance had been lowered by abnormal disturbances of metabolism." Many of the animals which later died had remained for months in an emaciated, dwarfed condition. This condition did not develop till some time after the operation. As stated above, all so-called runts were excluded at the beginning. The retarded development then must have been due to thyroid deficiency.

Even if the deaths had been due to infection of any kind it would still be necessary to show why these infections attacked only the oper-

ated animal. Such a result would still be attributable to disturbed metabolism or lowered resistance. Again the scattered distribution of deaths would contra-indicate the incidence of an infection.

That the deaths are primarily due to a thyroid rather than a parathyroid deficiency might be inferred from the longevity of many of the animals after operation. As in the previous research in the cases of very young operated animals which died two to four days after operation some evidences of tetany were observed, only three cases in the present experiment. However the general condition of the animals up to death indicates a thyroid deficiency. Many of the operated animals exhibited what might be termed myxedematous symptoms. In addition to a dull lethargic condition there was a marked difference in the state of the coats of the operated animals and the normals. This condition in the operated individuals varied from dirty yellow coats to lack of hair in many spots. Three very unusual conditions were noted which the writer has not seen mentioned elsewhere. The most striking was in some individuals a complete loss of hair along the midline of the back. This gave the white rat a weird appearance, due to the bright red skin along the back in contrast to the white coat elsewhere. The bare place ran along the back and top of the head, being widest at the head. This condition was detected in about twelve of the operated animals. It appeared about the second week after operation and disappeared within four weeks, new hair growing and the general condition of the animal improving. This subsequent reappearance of hair is in accordance with the findings of Cramer and McCall (2) who state that after thyroidectomy in rats there is at first a fall in metabolism which is later followed by a return to normal or nearly normal condition. In eight other operated animals there was a peculiar dirty, pinkish coloration on the top of the head forming a V-shaped pattern. This appeared about four months after the operation and upon animals in poor condition, and persisted until death. In other animals, chiefly in those operated animals which received kidney, the hair disappeared completely in large irregular patches about the body. As time went on this condition grew worse. Seven of the operated rats became blind in one eye and two blind in both eyes. Of these one was in the pituitary group and the others in those receiving normal diet. Blindness has frequently been described in connection with pituitary disorders.

SUMMARY

The results of the present series of experiments show:

1. That the effect of administration of anterior lobe of the pituitary to thyroidectomized rats tends to prolong life and to accelerate growth. This confirms the results stated in the previous paper.
2. That pituitary feeding also noticeably increases the rate of growth in normal rats.
3. Anterior lobe seems to exert more influence upon the growth of the normal males than that of the normal females.

The writer wishes to express his gratitude and appreciation of Prof. S. S. Maxwell's interest and advice during the entire research.

BIBLIOGRAPHY

- (1) LARSON: This Journal, 1919, xlix, 55.
- (2) CRAMER AND MCCALL: Quart. Journ. Exper. Physiol., 1918, xii, 81, 97.
- (3) HOSKINS AND HOSKINS: Endocrinology, 1920, iv, no. 1.
- (4) ALLEN: Anat. Rec., 1919, xv, 353.

THE INFLUENCE OF AN ALCOHOLIC EXTRACT OF THE THYROID GLAND UPON POLYNEURITIC PIGEONS AND THE METAMORPHOSIS OF TADPOLES¹

EMILY C. SEAMAN

*From the Department of Experimental Medicine, Cornell University Medical
College*

Received for publication May 26, 1920

It has been found by Eddy (1) and other workers (2) that water-soluble vitamins can be extracted from various animal tissues. A study was made in this laboratory to ascertain whether the thyroid gland contains these substances and what physiological action, if any, they possess.

I. EFFECT OF EXTRACT ON POLYNEURITIC PIGEONS

Fresh glands were ground in a meat chopper and extracted with 95 per cent alcohol made 0.8 per cent acid with HCl. To 1000 grams of the chopped glands were added 2 liters of the acid alcohol. The jars containing the material were shaken in a shaking machine for two or three hours, and allowed to stand forty-eight to seventy-two hours. The extract was filtered, the bulk of the alcohol distilled off and the residue evaporated to dryness before an electric fan. The dry residue was dissolved in hot water, filtered, evaporated to dryness a second time and re-dissolved in 500 cc. of hot water and filtered. This filtrate was a dark brown solution, yielding a positive reaction with Biuret and Millon reagent, and a strong deep blue color with the special phosphotungstic acid reagent, the color reaching its maximum density after standing from two to three hours. One cubic centimeter of the extract contained 0.05 mg. of iodine and 0.435 per cent of nitrogen. Later attempts to isolate the vitamin fraction failed to separate it from the one containing iodine. The vitamin quality of the extract was studied by the curative effects on polyneuritic pigeons and, as a control on the

¹ From the Johnston Livingston Fund for Experimental Therapeutics.

effect of the iodine content, the "residue"² as prepared in the laboratory was used in corresponding doses. In every case the residue failed to produce any curative effect on the polyneuritic pigeons, demonstrating that it was not the iodine content which produced results. The extract showed rapid and marked curative properties in every case in which the whole extract was used. Separate extracts from different lots of glands were made. It was found when the same proportions of glands and acid alcohol were used that the final solution was not always of the same strength. This strength was standardized according to the iodine content and the final solution either evaporated or diluted until 1 cc. contained 0.05 mgm. of iodine. There was a slight variation in the nitrogen content.

The extract was divided into four portions:

- a. The extract used as a whole.
- b. Precipitation by phosphotungstic acid to isolate vitamine fraction after the method of Funk.
- c. Extract treated with Lloyd's reagent and dry powder used.
- d. Portion reserved for experiments on metamorphosis of tadpoles.

Results

The whole extract when used on polyneuritic pigeons gave marked and rapid curative results. The extract which had been treated with phosphotungstic acid, barium sulphate, mercuric chloride and H_2S , as outlined by Funk and Eddy (1), gave no results in any case. The administration of the powder, obtained after shaking the extract with

² The thyroid "residue," as described in a previous communication from this laboratory, is the non-coagulable portion of a slightly alkaline extract of the glands after the nucleo-proteins have been removed. The hashed fresh glands are extracted for twenty-four hours at room temperature in tap water made faintly alkaline with NaOH. After straining through cheese-cloth, the filtrate is treated with 10 per cent acetic acid to precipitate the nucleoproteins. The acid filtrate is heated to boiling to remove the acid heat-coagulables; the filtrate from this made alkaline and again brought to boiling and filtered. This filtrate is neutralized and concentrated until 1 cc. of the filtrate contains 0.05 mg. of iodine. This thyroid "residue" produces, when injected into dogs, quite definite physiological reactions. In the stomach and pancreas it appears to stimulate the functions believed to be performed by the terminals of the gastric and pancreatic fibers of the vagus nerve. A neutral alcohol extract and the acidulated alcohol produce closely similar physiological effects, but clinically the acid alcohol extract does not seem as useful as either the neutral alcohol extract or the "residue."

Lloyd's reagent, gave positive results but the improvement was slower. The protocol for the administration of the whole extract given to polyneuritic pigeons is as follows:

Six pigeons were fed upon polished rice for weeks until extreme symptoms of polyneuritis occurred. Two pigeons were fed on normal pigeon food and used as controls. Two of the polyneuritic pigeons were given the "residue" by mouth in amounts corresponding to the iodine content of 4 cc. of the extract. In both cases no curative effect was produced in twenty-four hours after three doses of the residue. One pigeon who received only the residue died; the second, who received the residue and later the extract, survived.

With the four pigeons which were given the extract marked improvement was seen within six hours; demonstrating quite conclusively that the extract possessed curative properties for polyneuritic pigeons which the residue at this time did not possess.

Pigeon I. (Vitamine extract). Bird prostrate in cage and apparently dying. Weight dropped from 325 grams to 296 grams.

February 23, 10:00 a.m. 4 cc. extract by mouth
 2:00 p.m. Feathers smoother, bird moving head, and eyes brighter; 2 cc. of extract by mouth
 5:00 p.m. Bird crouched on legs, still unable to stand; 2 cc. extract given by mouth
 February 24, 9:00 a.m. Walked strongly about cage; 4 cc. extract given by mouth
 2:00 p.m. Bird apparently normal; flies across cage. When placed on floor of room, able to walk but totters a little after a dozen steps; 4 cc. extract given by mouth
 February 25, 9:00 a.m. Bird walked strongly about floor of room. Dosage discontinued

Pigeon II. (Vitamine extract)

February 25. Weight dropped from 300 grams to 232 grams. Marked symptoms of paralysis.

11:00 a.m. 3.5 cc. of extract
 4:50 p.m. Bird raising itself on legs if touched; 2 cc. extract given by mouth
 February 26, 10:00 a.m. Marked improvement; bird perched on food pan, feathers smooth and bird apparently normal. No further dosage
 March 15. Bird in good condition, flies and walks strongly. Put back on normal food

Pigeon III. (Vitamine extract)

February 25. Weight dropped from 257 grams to 221 grams. General paralysis.

11:00 a.m. 2 cc. extract given by mouth
 4:30 p.m. 4 cc. extract given by mouth

- February 26, 10:00 a.m. Marked improvement; 2 cc. extract given by mouth
 3:00 p.m. Bird normal; 2 cc. extract given by mouth
- February 27. Bird in such good condition, dosage discontinued
- Pigeon IV. (Vitamine extract)*
- February 27. Weight dropped from 295 grams to 213 grams. Total paralysis.
 9:00 a.m. 4 cc. extract given by mouth
 2:00 p.m. Bird crouched on legs, unable to stand; 2 cc. extract given by mouth
- February 28, 9:00 a.m. Bird walking about cage; 2 cc. extract given by mouth
- March 1. Bird walks strongly with head erect
 9:00 a.m. 2 cc. extract given by mouth
 2:00 p.m. Bird normal; dosage discontinued
- Pigeon V. (Residue)*
- February 26. Weight dropped from 312 grams to 241 grams. Extreme symptoms of polyneuritis.
 10:00 a.m. 4 cc. residue given
- February 27. 9:00 a.m. Bird dead
- Pigeon VI. (Residue and extract)*
- March 1. Weight dropped from 297 grams to 231 grams. Extreme symptoms of polyneuritis.
 9:00 a.m. 4 cc. residue given by mouth
 2:00 p.m. No results; 2 cc. residue by mouth
 5:00 p.m. No improvement; 2 cc. residue by mouth; 4 cc. extract given by mouth
- March 2. 9:00 a.m. Bird living and crouched on legs; 2 cc. extract given
 11:00 a.m. 2 cc. extract given
 2:00 p.m. Bird improved but totters about cage
 5:00 p.m. 2 cc. extract given
- March 3. 9:00 a.m. Bird tottering about cage; decidedly improved but not normal; 4 cc. extract given
 2:00 p.m. Bird walking normally; 2 cc. extract used.

Note. This bird never regained its normal vigor. It continued to improve and walked and flew about its cage but much of the time remained crouched down. After ten days it was put back on normal food, but even then showed less vitality than the other birds.

Conclusions

From the above experiment with polyneuritic pigeons it was demonstrated that the water extract from the thyroid glands made by 95 per cent alcohol made 0.8 per cent acid with HCl possessed the same curative property which has been attributed to water-soluble vitamins.

II. EFFECT OF ACID ALCOHOLIC THYROID EXTRACT ON METAMORPHOSIS OF TADPOLES

It has been found by Gudernatsch (3) and others that the metamorphosis of young tadpoles is hastened by feeding thyroid glands. Gudernatsch found that feeding the whole gland produced the hind legs in nine days after feeding, and the fore legs two days later. When these thyroid-fed tadpoles put out their anterior limbs and began to shorten their tails, they were eighteen to twenty days old. Swingle (4) obtained similar results by feeding iodoform, potassium iodide and iodine crystals. His most rapid results were obtained from the iodine crystals, limb buds appearing "in a few days." More tardy results were obtained with iodoform and potassium iodide.

It is claimed by Swingle and other workers that iodine is the active principle which hastens the metamorphosis of tadpoles, and this theory is generally accepted. In the following experiments done in this laboratory with the acid alcoholic extract, controls were made with iodine crystals, potassium iodide, thyroid nucleoprotein and the thyroid "residue" in amounts with corresponding iodine content.

Rana pipiens, approximately 15 mm. in length, were used and kept in tap water which was changed every day. To the fresh water were added the various substances in doses corresponding to 0.10 mg. of iodine a day. Each jar contained fifteen or twenty tadpoles and the experiments were repeated four times. A total of not less than sixty tadpoles was used for each substance, but out of this number at least fifteen died before complete metamorphosis. By far the most rapid results were obtained with the thyroid extract prepared by extracting the glands with 95 per cent alcohol made 0.8 per cent acid with HCl, as described in part I, and which proved to have the property of water-soluble vitamins. The limb buds appeared within eight hours, and complete metamorphosis with the bulging eyes and disappearance of the tail within four days. By the time complete metamorphosis had occurred in these specimens, only slight signs of limb buds appeared in the specimens treated with the iodine crystals, and no change occurred in the specimens fed the other substances. Negative results were obtained with the nucleoprotein and "residue," after continuing the feeding nearly three weeks. As the "residue" and alcoholic extract contained the same amount of iodine (1 cc. containing 0.05 mg. iodine) and no results were obtained with the residue or nucleoproteins, it is evident that the iodine content alone is not the only factor. It is



1



2



3



4



5



6



7

Fig. 1. Less than twenty-four hours
Fig. 2. Thirty-six hours
Fig. 3. Forty-eight hours
Fig. 4. Three days

Fig. 5. Four days
Fig. 6. Five days
Fig. 7. Control

very probable that the iodine of the thyroid gland persists in more than one combination. The alcoholic extract may have contained, and according to Kendall (5) did contain, a substance not present in the residue, but Kendall did not obtain any such rapid results in the metamorphosis of tadpoles after feeding them "thyroxin" as took place with this alcoholic extract.

The following table represents the condition of the specimens when the experiment was performed on tadpoles approximately ten days after development from the egg. All the specimens did not develop in exact uniformity of time, there being a lag of four to six hours in some, but all showed complete metamorphosis by the end of the fourth day.

Table of results

HOURS	CONTROL	CRYSTALS	POTASSIUM IODIDE	NUCLEO- PROTEIN	RESIDUE	ACID ALCOHOLIC EXTRACT
12						Appearance of limb buds
36						Growth of legs
48						Emaciation begun.
						Growth stopped
72		No increase in growth				Tail beginning to disappear
96	Growth	Limb buds				Complete metamorphosis

See figures.

Effect of age on metamorphosis

The above experiment was repeated with four sets of tadpoles of the same age but hatched from eggs procured from different stores. The results were the same in each experiment.

When the experiment was repeated two weeks later, when the tadpoles were a month old, the development was slightly retarded, complete metamorphosis occurring not later than six days. In the experiment with tadpoles two months after they had been hatched, complete metamorphosis took place within ten days. Fewer specimens died when the older tadpoles were used.

GENERAL CONCLUSIONS

It has been found that the thyroid gland, as well as other tissues of the body, contains water-soluble vitamins, the property being demonstrated by the curative action on polyneuritic pigeons.

It was also found that the acid alcoholic extract had a marked accelerating action on the metamorphosis of tadpoles, the most rapid development occurring in specimens two weeks after they had been hatched, and slightly slower development as the age increased.

BIBLIOGRAPHY

- (1) EDDY: *Journ. Biol. Chem.*, 1916, xxvii, 113.
- (2) OSBORNE AND MENDEL: *Journ. Biol. Chem.*, 1917, xxxii, 309.
- (3) GUDERNATSCH: *Amer. Journ. Anat.*, 1913-14, xv, 431.
- (4) SWINGLE: *Endocrinology*, 1918, ii, 283.
- (5) KENDALL: Lecture delivered before the Harvey Society, 1919.

THE ALKALI RESERVE IN EXPERIMENTAL SURGICAL SHOCK

BERNARD RAYMUND

From the Hull Physiological Laboratory, University of Chicago

Received for publication April 26, 1920

INTRODUCTION

The work herein reported was taken up in response to suggestions made by the Sub-Committee on Shock of the Committee on Physiology, National Research Council. Since the question of acidosis as a factor in shock became of such general interest, it seemed desirable to devote attention first to this, and leave for further consideration other factors, perhaps of equal importance. Hence this paper will be confined to the presentation of such data as have been accumulated in this laboratory with regard to the alkali reserve in shock.

Attention was first directed to the possible rôle of acidosis in the condition of shock by the claim of Spiro (1) in 1902 that the administration of hydrochloric acid to rabbits, or the intravenous injection of sodium hydrogen phosphate in dogs, was followed by "shock" effects. Howell (2) stated in 1903 that the intravenous injection of a 0.5 per cent sodium carbonate solution had a beneficial effect upon animals in shock but took the view (3) that this was due primarily to its action upon the heart. Apparently the question of its neutralizing effects did not enter into consideration. Dawson (4), using sodium bicarbonate after hemorrhage, reached the same conclusion. Seelig, Tierney and Rodenbaugh (5) using repeated small injections of sodium acid phosphate, stated that they were able to bring about a condition of rather profound shock in dogs, as judged by blood pressure readings. On the other hand, intravenous injections of sodium bicarbonate proved of decided benefit to animals in shock, but the authors judged that it acted directly upon the heart muscle. Yandell Henderson in 1910 (6) stated it to be his belief that the fatally rapid transudation of fluid from the veins in shock was due to the action of acidosis bodies upon the proteins of the tissues, causing them to imbibe water. He admitted

that the process was probably very complex. Crile attempted to show in 1915 (7) by histological methods that the effects of the injection of sodium acid phosphate upon brain, liver and adrenal cells were in all respects similar to those found after death from surgical shock, and that such post-operative lesions could be prevented by the previous injection of sodium bicarbonate. He declared (8) that the bases of the body are in many cases exhausted by acid products developed during operations and recovery thereby rendered impossible. Corbett however (9) was unable to demonstrate any increase in urinary ammonia during traumatic shock, nor any increase in the hydrogen ion concentration of the blood, and concluded that the relation of acidosis to shock was not clear.

At the time this work was begun, Cannon (10) had stated in an informal memorandum to the Committee on Physiology of the National Research Council that "it is probable that the acidosis is causally related to the clinical condition presented in shock. . . . Arterial pressure falls (vascular walls relax and cardiac contractions become less vigorous) when acid is injected experimentally into the blood vessels." Furthermore "a fairly close inverse relation exists between the degree of acidosis and the height of the arterial blood pressure—the more marked the acidosis the lower the pressure" and "as the degree of acidosis increases the general condition of the patient becomes worse—the greatest degree is found near death."

Early in the next year Cannon (11) reported work undertaken with Bayliss which seemed to show that "if the alkaline reserve is diminished by very slow injection of acid ($N/2$ HCl) into a vein, there is a fall of blood pressure to the shock level after the alkali reserve reaches a certain critical point (38 per cent in the cat). . . . Bruising or mashing the muscles of the hind legs is followed by a progressive fall in alkali reserve, and when the critical point is reached the blood pressure falls to the shock level." However in attempting to repeat the experiments with acid Bayliss (12) was unable to obtain the fall "except in cats obviously unhealthy."

Basing his assumptions upon this previous work Cannon (13) suggested that an excessive production after wounds of some acid metabolite and its distribution through the circulation might cause dilatation of the blood vessels, excessive lymph formation in all parts of the body and thus bring on exemia or shock. Similarly Henderson, Prince and Haggard (14) found that the carbon dioxide capacity of the whole blood could be greatly reduced by shock procedures and ventured the

opinion that the acidosis of shock and of ether anesthesia is compensatory to, or a result of, the acapnia produced by hyperpnoea. In his first paper in 1918 Cannon (15) while still emphasizing the importance of acidosis in shock does not assign to it a causative rôle, merely stating that the drop in alkali reserve under anesthesia is greater the less the original margin of safety in the individual, and that such cases showed an ominous fall of blood pressure when operated, especially if at the time in a state bordering upon shock. In subsequent papers (16), (17) he stated that "as the blood pressure falls there is a loss of the alkali reserve of the blood (acidosis) roughly corresponding to the drop in pressure." In other words, acidosis had been relegated to a position of secondary importance, although Cannon still maintained that the injection of sodium bicarbonate was of prophylactic value in cases of shock.

Practically the same conclusion as to the importance of acidosis in shock had been reached independently by Guthrie (18) and McElroy (19), although Henderson and Haggard (20), (21) stated that if in dogs under ether anesthesia "the level of CO_2 and alkali is reduced below the critical value lying between 33 and 36 volumes per cent of CO_2 , a condition of general depression of all functions results. . . . If the marked resistance to further depletion of the carbon dioxide capacity, which occurs at the critical level, is broken down and the CO_2 capacity further reduced, the result is a condition of vital depression from which the subject does not spontaneously recover. This condition may be termed *acapnial shock*." A low carbon dioxide capacity they regard as one of the definite criteria of shock. Subsequently Gesell (22) has shown that after either hemorrhage or visceral trauma, the reduction in the alkali reserve of the blood tends to be compensated for by a transfer of alkali from the tissues, and that the injection of $\text{N}/2$ HCl into normal animals is followed by indications of cardiac failure following a rise in arterial blood pressure. In this case also there is a passage of alkali from the tissues into the blood, so that successive doses of acid become less efficient in lowering the alkali reserve. Likewise Gasser and Erlanger (23) state that the alkali reserve does not show a sharp decline until after the animal is in a relatively advanced stage of shock, and that the reduced CO_2 capacity is the result, and not the cause of the fall in the arterial pressure.

CRITERIA OF SURGICAL SHOCK

Although the criteria for surgical shock are well established and quite generally recognized, most of the workers in the problem have shown a tendency to set some certain arterial blood pressure as the shock level, so-called, and to use this as a basis for determining at what time the animal passes into a state of shock, and to what degree. That the level of arterial pressure may be quite high when an animal is in true shock, and that the converse may just as readily be true, was pointed out by Meltzer (24) in 1908. This conception is given additional emphasis by Mann (25). He proposes as criteria of shock the following signs, which have become generally adopted: *a*, loss of sensibility; *b*, pallor of the mucous membranes; *c*, small, weak pulse; *d*, irregular, rapid, shallow or gasping respiration; and *e*, markedly lowered blood pressure (one-third to one-fourth of original level).

In view of the uncertainty as to what really constitutes a shock level of blood pressure, it seemed desirable on the whole to adopt these criteria as being the most satisfactory we possess, and in the experiments described in this paper they were constantly used in deciding whether or not the animal was in true shock. More emphasis was placed upon the first sign than upon the others, and in addition it was found that Guthrie's respiratory sign (26) often proved of value. This consists in a markedly prolonged inspiratory pause, and while not always present, is, when it appears, a reliable index, marking the beginning of the terminal respiratory paralysis.

EXPERIMENTAL PROCEDURE

Ether was used exclusively as the anesthetic, being given intratracheally and at such rate that the lid reflex was not abolished at any time during the experiment. In some experiments the patellar reflex was also used as an index of the depth of narcosis, although it was evident that it is neither as sensitive to the effects of ether nor, in the conscious dog, to those of prolonged low blood pressure, as the lid reflex. Thus in one experiment without ether and under local anesthesia, it was elicited after the corneal reflex had disappeared and when the heart was only beating with each occasional inspiration. That with the ordinary equipment it was possible in this way to keep the effects of the anesthetic at a minimum is shown in the protracted control experiments to be described later.

In order to obtain uniform results the dogs were not fed for eighteen hours previous to the experiment. Such exceptions as occur are noted. Determinations of the alkali reserve were made by Van Slyke's direct method (27). The blood was drawn either from the saphenous vein or, when the dogs were under ether anesthesia, from the femoral or carotid artery, oxalated, and immediately centrifuged for ten minutes, usually in a stoppered tube. Precautions to prevent the escape of carbon dioxide, such as the use of paraffin oil, were not taken. Peters (28) has stated that if the blood is centrifuged within fifteen minutes, the shifting of the carbonate into the erythrocytes does not occur, when the surface of the blood exposed to the air is small. In every instance the blood used in these determinations was centrifuged not more than ten minutes after being drawn. The plasma was saturated with alveolar air and analyzed in the usual manner. In a few cases it was allowed to stand over night in the refrigerator, but the analyses were checked by preserving a sample that had been analyzed beforehand and re-determining the alkali reserve the next morning. In no case could any change be detected. The results of the analyses, made in duplicate, were calculated to volumes per cent carbon dioxide at 0°, 760 mm. pressure, on the basis of saturation at 37°C., according to Van Slyke's formula. When parallel determinations were made on the whole blood the sample was divided into two portions, and while one was being centrifuged the other was saturated and analyzed. The reading so obtained was corrected for oxygen, etc., by the use of a few drops of 10 per cent sodium hydroxide solution.

Blood pressure and respiration were recorded in the usual manner. In most of the experiments shock was induced by a uniform technic, essentially that adopted by all workers in the field. After the blood pressure had reached a fairly stationary level, the abdomen was opened, the ether discontinued, the intestines removed and handled gently and continuously for fifteen minutes. Whereupon the guts were at once replaced and the abdomen closed securely with hemostats. From this point on the animal was kept warm, either by an electric light or by a heating pad, in order to eliminate the factor of lowered body temperature. If after a certain lapse of time, preferably an hour, the blood pressure showed no permanent lowering, another fifteen-minute period of trauma was resorted to as before. Occasionally the trauma had to be repeated several times before the animal showed any indication of shock; but except in one or two of the earlier experiments, the intestines were always replaced and not allowed to lie exposed to the air.

By closing the abdomen the degree of trauma can be much more accurately gauged, and there is furthermore no interference with the muscles of respiration.

In highly resistant animals there was sometimes resort to traction on the kidney, trauma to the liver or to the urinary bladder. These measures, especially the first, always caused a great disturbance in blood pressure. Such measures as clamping the inferior vena cava, occlusion of the portal system or of the abdominal aorta (29), (30), (31), seemed unnecessarily severe and were not used. Moderate trauma to the intestines in the manner described is sufficient to produce what appear to be the main clinical signs of shock, and the method can be controlled with fair accuracy.

In five of the experiments the procedure described above was followed, except that the entire gut was inflated with moist air at 40°C. to a pressure somewhat above the systolic blood pressure for a period of ten minutes. Five minutes were allowed for tying off the stomach and rectum, inserting the cannula in the caecum, and for deflating the gut and replacing it in the abdominal cavity. The guts were kept covered with hot towels throughout the entire period. This method is by far the most satisfactory of any that were tried, and makes it possible to control the degree of trauma with almost quantitative accuracy. By abolishing the circulation in the gut, asphyxia of the vessel walls and probably of the smooth muscle cells is set up, yet on the other hand the trauma is not so severe as to abolish the myenteric reflex. This is a very desirable feature, since it is doubtful if operative procedures in man ever completely do away with the reflex.

In seven experiments the method of Mann (32) was followed in inducing shock. All the structures in each limb with the exception of the supplying artery were tied off with iron wire ligatures. In this method the trauma is continuous throughout the experiment, but is possibly less severe than that involved in the foregoing procedures. Hence it is easier to follow the course of shock, and in this respect the method possesses certain advantages.

TYPES OF SHOCK

Naturally, if it is desired to compare the susceptibility of animals to trauma under various conditions, some certain basis for comparison must be found. By many workers in the problem the shock level of arterial blood pressure has been set at 50 mm. of mercury. But since

this figure is admittedly arbitrary, and since its adoption is not universal, it seemed inadvisable to compare the resistance of animals to trauma on the basis of the length of time, after beginning shock procedures, that the blood pressure remained above this level. As the only alternative it was necessary to select as a criterion of susceptibility the *survival time* of the animal, that is, the length of time it remained alive under the conditions of the experiment, after beginning traumatization.

Using this as a basis it was found that the animals studied fell into four general classes, that is, there were in all four *types of shock*, so-called. These were as follows:

Type I. With one or two exceptions, in the dogs showing this type of shock, only one fifteen-minute period of trauma was needed to set up a serious condition. Of a total of thirty-one dogs, five, or 16.1 per cent, showed type I.

Maximum survival time.....	2 hours, 10 minutes
Minimum survival time.....	0 hour, 52 minutes
Mean.....	1 hour, 27 minutes

Type II. Dogs showing this type of shock had to be given two fifteen-minute periods of trauma, but not more. Ideally these two periods should have been an hour apart, but this rule could not be followed very closely. When shock was produced by Mann's method (see above) the trauma was of course continuous. However I have included two of the dogs so traumatized in this class, since the survival time of each was found to lie within the proper limits. Of a total of thirty-one dogs, eight, or 25.9 per cent, showed type II.

Maximum survival time.....	2 hours, 47 minutes
Minimum survival time.....	1 hour, 20 minutes
Mean.....	2 hours, 9 minutes

It will be seen that type I and type II overlap somewhat and that the distinction between the two is in a measure arbitrary. This however is not the case with the last two types, which are clearly set off from the first two and from each other.

Type III. In this type are included the dogs which required as a rule more than two periods of trauma to seriously alter their condition. Of a total of thirty-one dogs, thirteen, or 41.9 per cent, showed type III. This includes three dogs traumatized by ligating the four limbs. All of these dogs died in true shock except one (exper. 90) which died of accidental hemorrhage. However this dog showed the same rate

of fall of blood pressure as the others in this class, previous to hemorrhage, and so it is included with them.

Maximum survival time.....	5 hours, 32 minutes
Minimum survival time.....	2 hours, 56 minutes
Mean.....	4 hours, 15 minutes

Type IV. Since the dogs in this class did not show typical shock, it is perhaps a misnomer to characterize this as one of the four types. However for the purpose of convenience this misnomer will be allowed to stand. Of a total of thirty-one dogs, five, or 16.1 per cent, showed type IV. This includes two dogs traumatized by Mann's method.

Maximum survival time.....	7 hours, 52 minutes
Minimum survival time.....	6 hours, 46 minutes
Mean.....	7 hours, 15 minutes

All of the dogs showing the first two types of shock gave a profound fall in blood pressure within thirty minutes after beginning the initial trauma. I had at first thought of using the degree of such fall as a criterion of susceptibility, and of determining the types of shock upon this basis. But the fall varied between such wide limits, 2 mm. to 102 mm., that this was impossible. Of the dogs exhibiting type III shock, all but three showed a fall in blood pressure (2 mm. to 49 mm.) thirty minutes after beginning initial trauma, while these three gave a rise in pressure (2 mm. to 36 mm.). However on classifying the data it was found that, without exception, every dog showing type IV shock gave a rise in pressure (3 mm. to 21 mm.) at the end of the thirty-minute period. This seems very significant, even granting that in type III shock such a rise of pressure may be seen. It shows that some notion of the course of shock may be obtained thus early in the experiment. This time rather than some other was chosen at which to make the observations on blood pressure, for the reason that at this time the wide fluctuations in blood pressure set up by the trauma have disappeared, while general and widespread damage to the circulation presumably has not had opportunity to occur.

The data summarized above are shown in graphical form in figure 1.

In the figure the heavily shaded areas represent periods wherein the intestines were traumatized. In the experiments in which the four limbs were tied off the block representing the survival time is left unshaded. In three such experiments where the ligatures were removed before the close of the experiment (indicated by the letter *A* in the

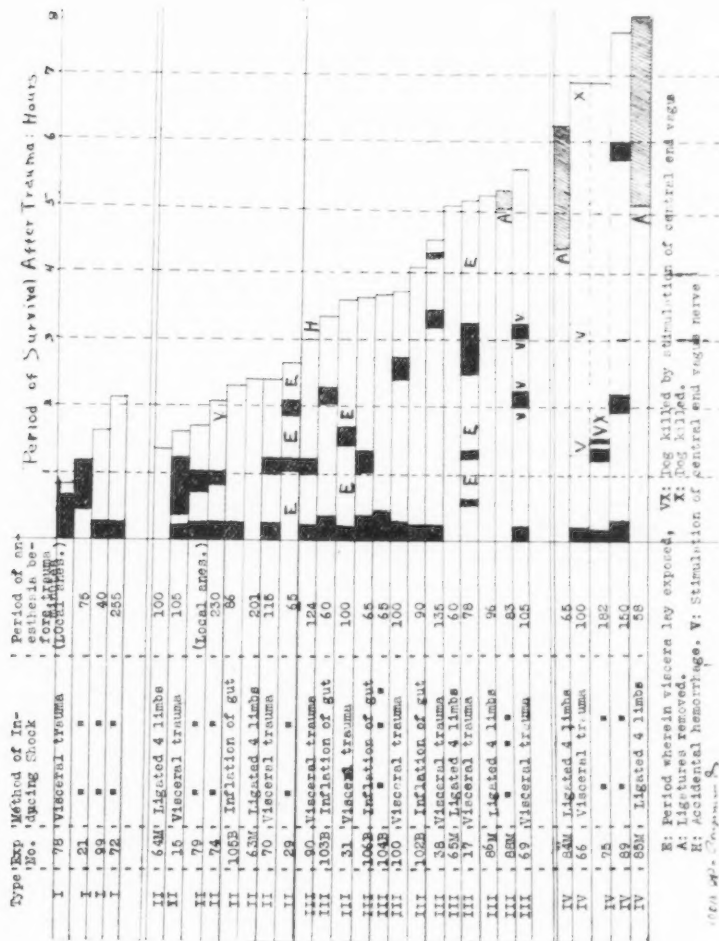


Fig. 1. Showing survival time of dogs after trauma and types of shock.

figure) the period after removal is indicated by cross-shading. Further, in these experiments trauma was considered to have been begun when both hind-legs had been ligatured off and before the fore-legs had been ligatured. In three early experiments in which the guts were allowed to lie exposed, such periods of exposure are indicated in the figure by the letter *E*. Prolonged stimulation of the central end of the vagus is indicated by the letter *V*, either in conjunction with trauma to the viscera, as in experiment 69, or alone. In experiment 75 where the dog was killed by such stimulation while the arterial blood pressure was still 123 mm., the fact is indicated by the letters *VX*. The letter *X*, experiment 66, indicates that the dog was killed with ether in order to bring the experiment to a close. It will be seen in column 4 of the figure that the length of the preliminary etherization period varied from one hour or less to more than four hours and fifteen minutes in one experiment. However it is improbable that this had much effect upon the survival of the animal (see control experiments, table 1, below).

Five experiments were performed with Dr. A. C. Ivy under local anesthesia, without the use of ether, aseptic precautions being observed. Blood pressure and respiration were recorded in three of these experiments; in all the plasma alkalies, red cell count, hemoglobin and corpuscle volume were determined at intervals. Only one of the dogs was strictly normal. The others had had the vagi and splanchnics severed above the diaphragm and the coeliac ganglion extirpated under asepsis some two or three weeks previously. However these with one exception have been included in the analysis given above. Since the experiments were few in number it is not proposed here to draw any conclusions from them save as regards survival time.

THE ALKALI RESERVE IN NORMAL DOGS NOT SUBJECT TO ETHER

Sixty-eight determinations of the plasma alkalies were made on forty dogs not subjected to ether. The results may be summarized as follows:

Maximum alkali reserve.....	59.5 volumes per cent (exper. 68)
Minimum alkali reserve.....	32.4 per cent (experiments 4 and 20)
Mean.....	43.4 per cent (68 determinations)
Mean heart rate.....	105
Mean respiration.....	31

The dog showing the maximum alkali reserve had been fed.

In fourteen dogs, eighteen determinations were made of the alkali reserve of the whole blood, as follows:

Maximum alkali reserve.....	55.6 per cent (exper. 101)
Minimum alkali reserve.....	36.6 per cent (exper. 92)
Mean, whole blood.....	47.0 volumes per cent
Mean heart rate.....	108
Mean respiration.....	23

None of these dogs had been fed. What effect feeding might have on the alkali reserve was not definitely determined, but from the data in hand there would seem to be no intimate relationship; that is, the alkali reserve did not invariably increase after feeding. Of course this would depend largely on the character of the food.

The figures given above agree with those found by Morriss (33) for plasma, but not with those cited by McElroy (19). Henderson, Prince and Haggard (14) give 48 volumes per cent as the normal reserve of the whole blood in the dog.

THE ALKALI RESERVE IN DOGS UNDER CONTINUOUS ETHER ANESTHESIA

In determining the effect of the anesthetic upon the alkali reserve, arterial plasma was used, except in the first two experiments where the analysis was made upon the plasma of venous blood throughout. Although comparison is made here between the alkali reserve of the normal venous plasma and that of the arterial plasma after etherization, the error introduced thereby is probably slight and no doubt constant. The results are given in table 1.

For the sake of convenience the etherization may be divided into four periods of unequal length, viz.: period 1, first 45 minutes of ether anesthesia; period 2, 45 minutes to 2 hours; period 3, 2 to 4 hours; period 4, beyond 4 hours. On this basis the data presented in the following table may be summarized briefly as follows:

Period 1. First 45 minutes of ether

Number of determinations.....	18
Maximum alkali reserve.....	41.9 per cent (experiment 43)
Minimum alkali reserve.....	19.8 per cent (experiment 54)
Mean.....	33.9 volumes per cent
Maximum fall from normal.....	25.1 per cent (experiment 54)

Minimum fall.....	1.3 per cent (experiment 46)	
Mean fall.....	10.5 per cent	
Mean heart rate.....		189
Mean increase in same.....		91
Mean respiration.....		64
Mean increase in same.....		31

Period 2. Ether, 45 minutes to 2 hours

Number of determinations.....		18
Maximum alkali reserve.....	35.3 per cent (exper. 30)	
Minimum alkali reserve.....	16.4 per cent (see note 2, above)	
Mean.....	28.0 volumes per cent	
Maximum fall from normal.....	34.4 per cent (see note 3, above)	
Minimum fall from normal.....	4.0 per cent (exper. 30)	
Mean fall.....	15.8 volumes per cent	
Mean heart rate.....		151
Mean increase in same.....		45
Mean respiration.....		69
Mean increase in same.....		26

Period 3. Ether, 2 to 4 hours

Number of determinations.....		5
Maximum alkali reserve.....	31.9 per cent (exper. 96)	
Minimum alkali reserve.....	19.5 per cent (exper. 68)	
Mean.....	26.1 volumes per cent	
Maximum fall from normal.....	40.0 per cent (exper. 68)	
Minimum fall from normal.....	13.8 per cent (exper. 96)	
Mean fall.....	22.5 volumes per cent	
Mean heart rate.....		159
Mean increase in same.....		53
Mean respiration.....		62
Mean increase in same.....		31

Period 4. Ether, 4 hours plus

(See data for experiments 91, 92, 96, table 1)

THE ALKALI RESERVE IN DOGS AFTER TRAUMA UNDER LOCAL ANESTHESIA.
NO ETHER

The effects of visceral trauma upon the plasma alkalies when only local anesthesia was used are summarized below in table 2. These experiments were performed by Dr. A. C. Ivy and the writer upon one

TABLE I

Showing the effects of ether anesthesia upon the alkali reserve of the plasma in dogs

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE		HEART RATE	RESPIRA- TION
				Dias- tolic	Systolic		
		<i>per cent</i>	<i>per cent</i>	<i>mm.</i>	<i>mm.</i>		
9	Normal	50.7				90	28
	15 min. ether	37.6	13.1			210	70
10	Normal	47.5				90	23
	15 min. ether	40.4	7.1			264	60
11*	Normal	45.7				98	28
	15 min. ether	38.1	7.6			248	60
46	Normal	38.5				104	16
	20 min. ether	37.2	1.3			168	48
44	Normal	45.8				72	12
	25 min. ether	35.6	10.2			132	60
12*	Normal	44.3				102	24
	35 min. ether	38.1	6.2			244	40
90	Normal	40.9				80	6
	38 min. ether	30.5	10.4	146	155	140	56
70	Normal	51.0				60	16
	45 min. ether	36.2	14.8	140	150	132	56
69	Normal	44.9				120	24
	50 min. ether	30.0	14.9	123	131	150	64
29	Normal	36.6				75	24
	55 min. ether	23.3	13.3	102	109	96	72
66	Normal	51.6					
	55 min. ether	34.2	17.4	126	138	122	66
56	Normal	37.6				78	16
	56 min. ether	27.1	10.5	111	118	150	96
31	Normal	49.4				96	15
	75 min. ether	28.7	20.7	94	104	132	44

TABLE 1—*Concluded*

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE		HEART RATE	RESPIRA- TION
				Dias- tolic	Systolic		
		<i>per cent</i>	<i>per cent</i>	<i>mm.</i>	<i>mm.</i>		
55†	Normal						
	30 min. ether	27.7				204	44
	70 min. ether	16.4			60	174	84
30	Normal	39.3				132	56
	5 min. ether	30.0	9.3			228	66
	75 min. ether	35.3	4.0			180	38
54	Normal	44.9				108	20
	25 min. ether	19.8	25.1			180	52
	90 min. ether	28.2	16.7	115	124	144	76
38	Normal	43.0				72	56
	45 min. ether	31.5	11.5			186	72
	90 min. ether	24.0	19.0	147	153	180	72
60	Normal	40.0				130	80
	50 min. ether	34.3	5.7	124	130	144	108
	85 min. ether	29.6	10.4	118	125	126	104
40	Normal	39.3				108	174
	10 min. ether	31.9	7.4			144	68
	109 min. ether	28.2	11.1	151	154	141	102
43	Normal	44.9				144	48
	45 min. ether	41.9	3.0			144	48
	150 min. ether	32.8	12.1	134	138	192	76
89	Normal	40.4				120	20
	43 min. ether	31.9	8.5	109	121	140	72
	110 min. ether	26.2	14.2	109	125	150	68
14*	Normal	45.3				102	20
	7 min. ether	28.1	17.2			192	92
	56 min. ether	31.9	13.4			162	88
	113 min. ether	29.0	16.3			168	76
67	Normal	41.9				132	35
	58 min. ether	29.3	12.6	92	96	132	56
	120 min. ether	23.7	18.2	94	97	180	38
	180 min. ether	21.4	20.5	55	59	180	64

TABLE 1—Continued

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE		HEART RATE	RESPIRATION
				Diastolic	Systolic		
		<i>per cent</i>	<i>per cent</i>	<i>mm.</i>	<i>mm.</i>		
68‡	Normal	59.5				84	32
	40 min. ether	35.7	23.8	98	104		
	88 min. ether	27.1	34.4	59	69	132	84
	145 min. ether	19.5	40.0	54	58	180	52
	205 min. ether	22.3	37.2	26	30	102	6
91	Normal	51.0				88	16
	50 min. ether	33.7	17.3	128	131	124	40
	112 min. ether	30.5	20.5	119	122	176	40
	171 min. ether	29.0	22.0	119	121	148	64
	230 min. ether	31.5	19.5	124	126	144	60
	295 min. ether	35.3	15.7	126	128	106	48
	350 min. ether	33.4	17.6	119	122	120	58
	490 min. ether	26.3	24.7	77	79	118	48
	595 min. ether	25.4	25.6	37	38	Dying	
92§	Normal	45.7				108	44
	93 min. ether	30.0	15.7	108	118	120	40
	213 min. ether	28.7	16.0	82	102	128	52
	349 min. ether	24.0	21.7	94	100	128	52
	422 min. ether	26.8	18.9	86	90	164	52
	530 min. ether	26.8	18.9	68	72	160	68
	650 min. ether	30.9	14.8	58	62	176	72
96§	Normal	45.7				120	28
	60 min. ether	34.7	11.0			144	60
	120 min. ether	33.8	11.9			128	48
	240 min. ether	31.9	13.8			160	76
	300 min. ether	33.8	11.9			156	76
	360 min. ether	35.7	10.0			160	88
	420 min. ether	35.7	10.0			168	84
	480 min. ether	35.7	10.0			184	92
	600 min. ether	31.9	13.8			160	120

* Experiments 9, 10, 11, 12 and 14 performed on the same dog.

† Effects of over-etherization shown in experiment 55.

‡ Accidental hemorrhage in experiment 68.

§ Experiments 92 and 96 were aseptic ether controls performed with Mr. C. F. G. Brown assisting. Both dogs lived 12 hours after concluding the experiment.

normal dog and upon four dogs that had had the splanchnics and vagi sectioned in the thorax and the coeliac ganglion evulsated. The operations were performed aseptically two to three weeks previous to the time at which the experiments were conducted, and the dogs had recovered completely.

TABLE 2

*Showing the effects of visceral trauma upon the alkali reserve of the plasma in dogs
Local anesthetic. No ether*

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE, MEAN	HEART RATE	RESPI- RATION	BODY TEMPER- ATURE
		<i>per cent</i>	<i>per cent</i>	<i>mm.</i>			<i>°C.</i>
78	Normal	41.9		96	186	16	37.0
Type I	46 minutes*	32.4	9.5	60†	60	12	39.1
77	Normal	40.9			116	32	39.6
Type III	57 minutes*	35.3	5.6		162	26	39.7
	120 minutes*	23.0	17.9	+	156	20	39.0
79	Normal	40.0		124	80	14	39.1
Type II	53 minutes*	25.8	14.2	48	144	34	38.7
	93 minutes*	22.1	17.9	42†	124	20	38.6
80	Normal	37.2		164	140	12	38.3
Type I	33 minutes*	30.9	6.3	48	170	28	38.1
	69 minutes*	29.0	8.2	42	134	32	
	135 minutes*	22.3	14.9	47†	140	32	37.0
76	Normal	35.3			144	44	38.9
Type II	55 minutes*	30.9	4.4		162	32	37.7
	99 minutes*	24.2	11.1	†	80	12	36.4

* Lapse of time after beginning *initial* trauma.

† Animal in shock at time of observation.

Analyses were made on the plasma of blood drawn by syringe from the inferior vena cava or external jugular vein. The normal dog (exper. 78) was in a state of profound shock at the end of the first forty-five minutes after beginning the initial, and only, period of trauma. As shown in the table the alkali reserve of the plasma had fallen only 9.5 volumes per cent and was in fact no lower than the minimal value for the normal dog. This was a very clear case of type I shock. Of

the other four dogs, determinations made before the signs of shock appeared, gave a maximum fall from normal of 14.2 volumes per cent (exper. 79), and a minimum of 4.4 volumes per cent (exper. 76). The average fall for the four, within the first hour after beginning the initial trauma, was 8.1 volumes per cent. As will be seen, the reading in experiment 77 was quite above the normal minimum for the normal dog, namely 32.4 volumes per cent, while the other three gave readings not far below this point.

When we come to the final determinations, however, when all the dogs showed definite signs of shock, matters are somewhat altered. Thus two dogs show a fall from the normal alkali reserve of 17.9 volumes per cent (experiments 77 and 79). The average fall for the four is 15.2 volumes per cent. All show readings below the mean value found after two to four hours of continuous ether anesthesia (26.1 volumes per cent). There appears to be no relation between the type of shock and the degree of alkali depletion. However the data give an indication of what trauma, uncomplicated by ether anesthesia, will accomplish toward setting up a state of acidosis in the dog. It is seen further that until shock actually appears, the alkali reserve remains well above what may be called the limit of safety. The acidosis is certainly not marked. The changes in heart rate and respiration are neither marked nor consistent. As many dogs showed a decrease in both as showed an increase.

It should be emphasized that the marked fall in alkali reserve occurred only after the dogs were in a state of shock. The slight fall observed in the first hour, then, was due to local changes set up by the trauma and not to any circulatory failure. That the onset of acidosis is gradual is revealed in experiment 78, where the dog died from shock before the alkali reserve had fallen even below the minimum normal value.

THE ALKALI RESERVE IN THE ETHERIZED DOG AFTER TRAUMA

The fall in alkali reserve in anesthetized dogs following trauma can best be illustrated by summarizing representative experiments. The data for four such experiments, illustrating the four types of shock, are given in table 3.

The results given in the table above are shown graphically in figures, 2, 3, 4, 5A and 5B.

The data in the above table agree satisfactorily with those presented in table 2. As before, the alkali reserve of the plasma remained prac-

TABLE 3

Showing the alkali reserve after trauma in etherized dogs

EXPERIMENT NUMBER	CONDITION	ALKALI RE- SERVE	FALL FROM NOR- MAL	BLOOD PRESSURE		HEART RATE	RESPI- RATION	BODY TEMPER- ATURE
				Diastolic	Systolic			
		<i>per cent</i>	<i>per cent</i>					<i>°C.</i>
99 Type I	Normal	43.8				132	28	
	62 min. ether	20.2	23.6	153	165	188	61	41.0
	Obs.* 30 min.	21.1	22.7	96	103	160	64	42.0
	Obs.* 95 min.	20.2	23.6	30†				42.0
29 Type II	Normal	36.6				75	24	
	55 min. ether	23.3	13.3	102	109	96	72	
	Obs.* 55 min.	23.3	13.3	64	78	180	48	38.9
	Obs.* 95 min.	17.8	18.8	47	51	180	80	36.8
	Obs.* 127 min.	16.6	20.0	50	53	144	52	35.6
	Obs.* 170 min.	15.7	20.9	29	32†	108	32	
69 Type III	Normal	44.9				120	24	
	50 min. ether	30.0	14.9	123	131	150	64	
	Obs.* 20 min.	27.1	17.8	104	107	180	68	
	Obs.* 78 min.	27.1	17.8	94	98	168	40	
	Obs.* 135 min.	26.2	18.7	67	74	120	36	
	Obs.* 200 min.	15.9	29.0	55	61	126	40	
	Obs.* 255 min.	13.0	31.9	51	58	132	32	
	Obs.* 325 min.	17.8	27.1	20†		52	3	
66 Type IV	Normal	51.6						
	55 min. ether	34.2	17.4	126	138	122	66	
	Obs.* 35 min.	29.4	22.2	126	141	120	72	
	Obs.* 65 min.	30.3	21.3	96	111	150	76	
	Obs.* 115 min.	29.4	22.2	77	84	144	72	
	Obs.* 175 min.	26.5	25.1	78	84	150	78	
	Obs.* 242 min.	22.6	29.0	79	96	192	60	
	Obs.* 310 min.	23.6	28.0	46	53			
	Obs.* 382 min.	32.3	19.3	58	67	126	30	No shock

* Observation taken so many minutes after beginning the initial trauma.

† Observation taken when the animal was in shock.

tically unaffected in this case at the level to which it was brought by etherization, until the blood pressure had fallen; and only showed a significant reduction after the condition of the dog had become serious.

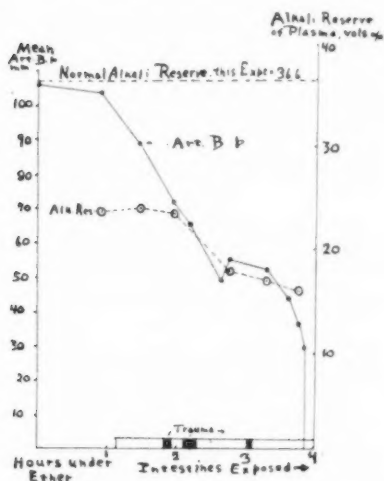


Fig. 2. Experiment 29. Showing the arterial blood pressure and the alkali reserve in a case of type II shock.

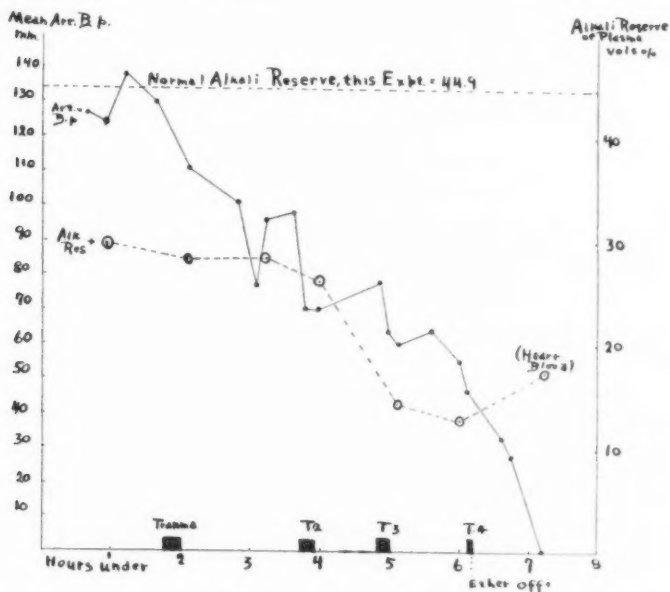


Fig. 3. Showing arterial blood pressure and alkali reserve in experiment 69. Type III shock.

Naturally there were exceptions. In experiment 90 (not shown) for instance, the alkali reserve of the plasma fell to 14.3 volumes per cent while the blood pressure was still 83 to 90 mm. The data also show that as the condition of the dog becomes worse, the heart rate decreases. I have never seen in the dog a case of cardiac shock, as described by Howell (2), (3), result from visceral trauma. At the same time the

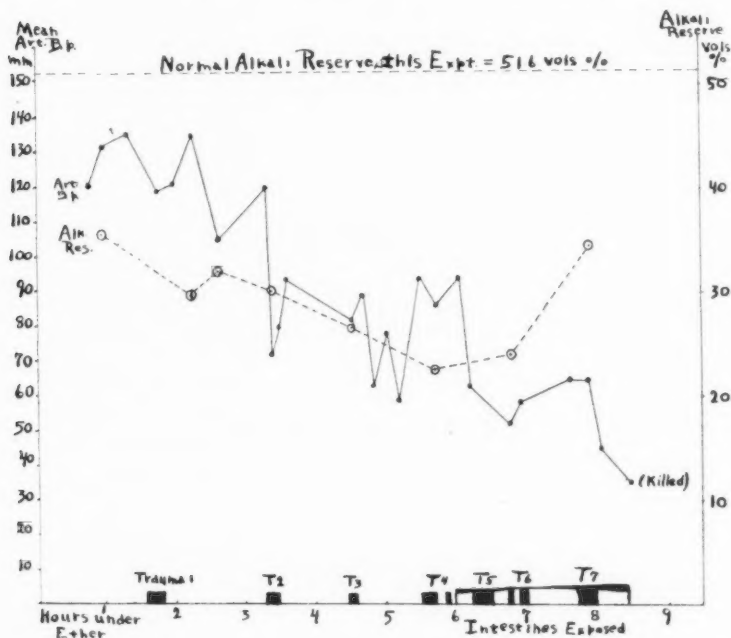


Fig. 4. Showing arterial blood pressure and alkali reserve in experiment 66. Type IV shock.

respiration rate usually decreased. Hence the terminal rise frequently observed in the alkali reserve of the plasma.

Although the data tend to show a correlation between the type, *i.e.*, the severity of shock and the fall in the alkali reserve, it appears doubtful, considering the experiments as a whole, whether this is at all close. True, the dogs exhibiting type IV shock were characterized by a remark-

ably constant alkali reserve. In these dogs compensation, such as by a decrease in the rate of respiration, etc., was no doubt very perfect, even when the blood pressure had fallen markedly. On the other hand, as regards types II and III, the distinction between the two could not be drawn upon the basis of the alkali reserve. Thus the lowest level to which the plasma alkalies were carried by this method, namely 13 volumes per cent (exper. 69, above) was in a case of type III shock.

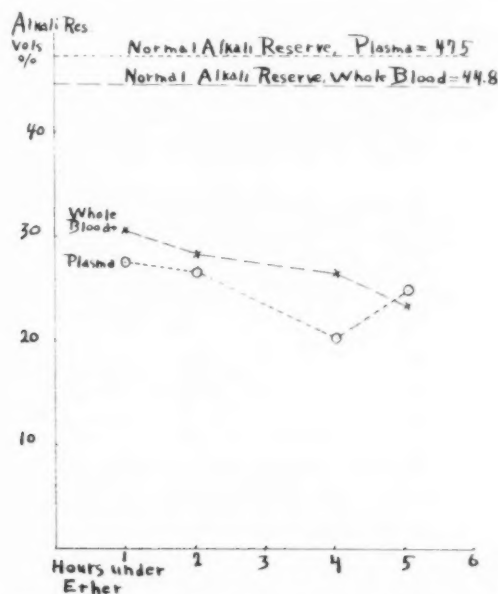


Fig. 5a. Showing alkali reserve of plasma and of whole blood. Aseptic ether control for experiment 99. October 30, 1919.

Again, selecting readings in the first hour after beginning the initial trauma, the alkali reserve in experiment 38 was 18.5 volumes per cent, as compared with 23.3 volumes per cent in experiment 29, although the type of shock in the first case was of the third order while in the second the dog showed type II shock. The blood pressure readings taken at the same time were 94-101 and 64-78 mm. respectively. However, it would be impossible on the basis of the data in hand to assign to any given range of arterial pressures definite values for the

plasma alkalis. The venture was made and proved utterly hopeless. Nor is there any critical level of blood pressure at which a marked decline of the alkali reserve is to be expected. The condition of the animal, in short, cannot be gauged by its alkali reserve alone.

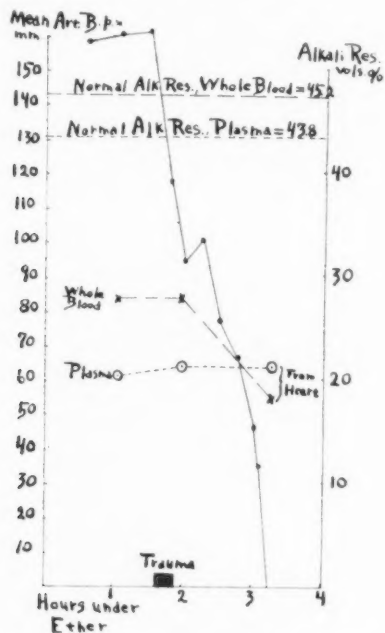


Fig. 5b. Showing arterial blood pressure, alkali reserve of plasma and of whole blood in experiment 99. Type I shock. November 6.

THE ALKALI RESERVE IN CONTROLLED SHOCK EXPERIMENTS

The accuracy of the conclusions reached above can be best borne out by presenting the protocol of one of a series of controlled shock experiments performed with the assistance of Mr. C. F. G. Brown. Dogs were kept under ether anesthesia for considerable periods of time, the anesthetic being administered by intubation. Blood pressure tracings were taken in only one experiment, but blood was drawn from the femoral artery with aseptic precautions at regular and rather frequent intervals. At the close of the experiment the dogs were sewed

up and allowed to recover. At the end of a week shock was instituted in the customary manner.

Protocol. Experiment 95, October 31, 1919. Aseptic ether control. Dog 56, male, 15 kilos. Fed.

TIME	ALKALI RESERVE		HEART RATE	RESPIRATION	BODY TEMPERATURE	PROCEDURE
	Blood	Plasma				
<i>a.m.</i>	<i>vol. per cent</i>	<i>vol. per cent</i>			<i>°C.</i>	
8:40	41.0	40.9	96	16		Sample, left saphenous
9:05						Ether, intubated, heat
10:05	28.3	26.8	140	40		Sample, rt. fem. art.
						Knee jerk positive
11:05	27.4	24.9	140	44		Knee jerk positive
<i>p.m.</i>						
12:05	25.6	25.8	142	36		Knee jerk positive
12:30						Vomited
1:05	28.3	22.1	136	68		Very excited
2:05	28.5	23.0	156	40		Lid reflex negative
3:05	27.5	24.0	152	60		Lid reflex positive
4:05	25.8	22.3	152	36		Lid reflex positive
4:35			140	48	35	Lid reflex positive
5:05	25.7	18.3	156	36		Lid reflex negative
5:35			160	64		Lid reflex positive
6:20					37	Sewed up, ether off

The lid reflex was positive except where otherwise indicated. The dog was under ether 9 hours and 25 minutes. Recovery was good and the dog ate the next morning. After the lapse of a week shock was instituted as shown in the protocol of experiment 100.

The lid reflex was positive throughout the experiment. The ether was disconnected during each period of trauma. On section the heart was found contracted, a further proof, if any were necessary, that in spite of the dog's great excitability the ether had been kept at a minimum level. The total survival time after beginning the initial trauma was three hours and forty-five minutes. Hence according to the classification adopted this was a case of type III shock, there being two periods of trauma, two hours and thirty minutes apart.

It should be noted that the lowest point to which the alkali reserve of the plasma fell, namely 17.7 volumes per cent, was only 0.6 volume per cent below the lowest point reached in the control experiment, and that this value, 18.3 volumes per cent, was perfectly compatible with

Protocol. Experiment 100, November 6, 1919. Shock experiment. Dog 56, male. 19.1 kilos. Not fed.

TIME	BLOOD PRESSURE		ALKALI RESERVE		HEART RATE	RESPIRATION	BODY TEMPERATURE	PROCEDURE
	Dias-tole	Systole	Blood	Plasma				
<i>a.m.</i>	<i>mm.</i>	<i>mm.</i>	<i>vol. per cent</i>	<i>vol. per cent</i>			<i>°C.</i>	
9:10			49.4	42.8	80	12		Normal venous sample
9:35								Ether, intubated, heat
10:28	109	127	31.8	31.5	140	44	39.5	Sample, left fem. artery
11:10	101	121	30.8	23.0	144	68	39.0	
11:15	94	113						Trauma, 15 min.
11:30	66	89						Closed up, heat
11:55	84	100			160	68	38.0	
<i>p.m.</i>								
12:10	78	90	29.9	26.8	184	80	38.5	
1:10	78	94	30.7	28.7	156	48	39.3	
1:45	89	109					39.5	Trauma, 15 min.
2:00	61	79			160	32	39.8	Closed up, heat
								Myenteric reflex positive
2:15	49	63	28.9	17.7	160	36	39.8	
2:25	53	67			176	28	39.0	Ether disconnected,
								breathing with diffi-
								culty, in shock
2:45	26	34	19.7	18.0	96	24	38.5	Respiration spasmodic,
								"shivery"
3:00								Dead

life. At the time, however, that the low reading was obtained in the shock experiment, the dog was already moribund, with an arterial blood pressure of 49 to 63 mm. Obviously this fact could never have been ascertained by a consideration of the plasma alkali alone. Furthermore the alkali reserve of the whole blood at this time was even slightly higher than in the control experiment at the same hour, namely four hours, forty minutes after beginning ether anesthesia. Bayliss (34) has shown that in cats a reduction of the alkali reserve of the plasma to a level as low as 5 volumes per cent by the intravenous injection of acid does not prevent the quick and apparently complete recovery of the animal. Thus the conclusion is inescapable that there is no critical level of alkali reserve, at least in the dog and cat.

ATTEMPTS TO REPRODUCE TRAUMATIC SHOCK BY INTRAVENOUS INJECTION
OF ACIDS AND ACID SALTS

Since the claim put forward by Cannon (11) that the blood pressure in cats can be brought to the shock level by the intravenous injection of $N/2$ HCl has been effectually disproved (34), it seems unnecessary to give an extended account of the experiments conducted along this line in connection with the present paper.

Intravenous injections of sodium acid phosphate in concentrations of 0.15 to 0.25 molar were made in thirteen dogs and two cats. Five cats and three dogs were given intravenous injections of $N/4$ HCl. While the injection volume was larger than if half-normal acid had been used, still it is doubtful if there was any significant elevation of blood pressure in consequence of this. Since the acid phosphate was quite ineffective in lowering the alkali reserve of the plasma to any extent, only the results obtained with hydrochloric acid will be given here.

The injection volume used in dogs varied between 6.9 and 42.3 cc. per kilo. All the cats were given 10 cc. per kilo. The lowest point to which the alkali reserve of the plasma was brought in the dog was 11.0 volumes per cent, from an initial normal value of 44.9 volumes per cent. Of this fall 16.7 volumes per cent were due to etherization, and the acid caused a further fall of 17.2 volumes per cent. There were signs of cardiac failure before death and on section the lungs were found to be intensely edematous. Signs of shock were conspicuous for their absence. The blood pressure on beginning the injection was 118-125 mm. After the alkali reserve had fallen to its lowest point the blood pressure remained above 90 mm. for forty minutes and at death was still 57 mm. Fourteen and one-tenth cubic centimeters of $N/4$ HCl were given per kilo.

The lowest point to which the plasma alkalies fell in the cat was 10.7 volumes per cent, from a value of 34.3 obtained an hour and three quarters after etherization. Of this fall 15.0 volumes per cent were due to the ether. The dose of $N/4$ HCl was 10 cc. per kilo. The blood pressure was fluctuating but rose from a level of 78-80 mm. at the time the last blood sample was drawn to 81-95 mm. and then fell rapidly to zero. There were no signs of cardiac failure; death occurred in apnoea. There was no hyperpnoea at any time nor any signs of shock, but the possibility of intravascular clotting and embolism was not excluded. Observe that in this cat the alkali reserve was at all times below the critical level of 38 volumes per cent cited by Cannon (11).

Summarizing, it can be said that none of the animals showed any signs of shock; in fact up to the point of death they were all unusually active. There were strong evidences of cardiac failure in the dogs, but whether it occurred in the cats is doubtful. That the oxygenation of the blood was markedly interfered with is shown in experiment 60. After the alkali reserve had been lowered from an initial normal value of 40 to 11.5 volumes per cent, the blood pressure was still 111-126 mm. The injection of acid was continued until the dog had been given 42.3 cc. per kilo of $N/4$ HCl. The blood pressure fell rapidly from 98-105 to about zero. On drawing a sample of blood from the heart it was found to be quite black and could not be oxygenated at all. The spectroscope revealed reduced hemoglobin only; there was no methemoglobin nor acid hematin. The alkali reserve of the plasma was negligible. In one cat in which death occurred very suddenly on injecting acid, the blood in the right ventricle was found to be coagulated immediately at the close of the experiment.

SUMMARY AND CONCLUSION

1. The dogs used in these experiments are classified on the basis of the length of survival after beginning the initial trauma. This makes unnecessary the arbitrary designation of any given arterial pressure as the shock level.
2. On this basis four general *types of shock* were made out, ranging from the more severe characterized by sudden onset and death, to that in which few or none of the cardinal signs of shock were observed.
3. The larger number of animals showed the intermediate types of shock and lived on the average from two hours, nine minutes, to four hours, fifteen minutes (types II and III) after beginning the initial trauma.
4. The average normal alkali reserve of the venous plasma in the dog was found to be 43.4 volumes per cent, sixty-eight determinations. The values ranged from 32.4 volumes per cent to 59.5. The average for whole blood was 47.0 volumes per cent, eighteen determinations. The maximum reading was 55.6, the minimum 36.6 volumes per cent.
5. In dogs under ether anesthesia the mean value fell to 33.9 volumes per cent. As anesthesia was protracted the mean alkali reserve fell to 28.0 volumes per cent (forty-five minutes to two hours) and finally to 26.1 volumes per cent (two to four hours). As etherization was continued beyond this point the mean fall in alkali reserve per unit of time was seen to decrease.

6. Five dogs traumatized under local anesthesia alone showed no striking fall in the alkali reserve of the plasma until their condition had become quite serious. No level of blood pressure could be set as critical in this respect. Under the conditions of the experiments the average fall from the normal reading was 15.2 volumes per cent.

7. Substantially the same was shown by eleven dogs which were traumatized under ether anesthesia. When shock ensued suddenly the fall in the alkali reserve of the plasma was relatively insignificant. When shock was late in appearing, the plasma might show a high alkali reserve for some time after the animal had become practically moribund. There was apparently no correlation between the type of shock and the degree of alkali depletion. The condition of the animal could not be gauged by its alkali reserve.

8. In several experiments it was found that the alkali reserve of the plasma and of the whole blood fell no lower in profound shock than in aseptic ether controls performed on the same dogs a week previous to the experiment, and from which they recovered rapidly and completely. If there is a critical level of alkali reserve it was not discovered.

9. Intravenous injections of N/4 HCl and of isotonic acid phosphate solutions did not produce shock or anything resembling this condition in either dogs or cats.

The writer desires to thank Dr. A. J. Carlson, Dr. A. B. Luckhardt and Dr. A. C. Ivy for their kind advice and many suggestions, and Mr. C. F. G. Brown for his assistance in the laboratory.

BIBLIOGRAPHY

- (1) SPIRO: Hofmeister's Beitr. 1902, i, 269.
- (2) HOWELL: Contributions to medical research, 1903, 57.
- (3) HOWELL: Amer. Med., 1904, vii, 482.
- (4) DAWSON: Journ. Exper. Med., vii, 1.
- (5) SEELIG, TIERNEY AND RODENBAUGH: Amer. Journ. Med. Sci., 1913, cxlvi, 195.
- (6) HENDERSON: This Journal, 1910, xxvii, 152.
- (7) CRILE: Ann. Surg., 1915, lxii, 257.
- (8) CRILE: Can. Med. Assoc. Journ., 1915, v, 1.
- (9) CORBETT: Journ. Amer. Med. Assoc., 1915, lxx, 380.
- (10) CANNON: Memorandum to the Sub-committee on Shock, Committee on Physiology, National Research Council, August 25, 1917.
- (11) CANNON: Ibid., February 25, 1918.
- (12) BAYLISS: Intravenous injections in wound shock, London, 1918, 60.
- (13) CANNON: Memorandum to the Sub-committee on Shock, Committee on Physiology, National Research Council, March, 25, 1918.

- (14) HENDERSON, PRINCE AND HAGGARD: *Journ. Amer. Med. Assoc.*, 1917, *lxix*, 965.
- (15) CANNON: *Journ. Amer. Med. Assoc.*, 1918, *lxx*, 531.
- (16) CANNON: *Journ. Amer. Med. Assoc.*, 1918, *lxx*, 611.
- (17) CANNON: *This Journal*, 1918, *xlvi*, 544.
- (18) GUTHRIE: *This Journal*, 1918, *xlvi*, 544; *Arch. Int. Med.*, 1918, *xxii*, 1.
- (19) McELLROY: *Journ. Amer. Med. Assoc.*, 1918, *lxx*, 846.
- (20) HENDERSON AND HAGGARD: *Journ. Biol. Chem.*, 1918, *xxxiii*, 345.
- (21) HENDERSON AND HAGGARD: *Journ. Biol. Chem.*, 1918, *xxxiii*, 365.
- (22) GESELL: *This Journal*, 1919, *xlvi*, 468.
- (23) GASSER AND ERLANGER: *This Journal*, 1919, *i*, 104.
- (24) MELTZER: *Arch. Int. Med.*, 1908, *i*, 571.
- (25) MANN: *Johns Hopkins Hosp. Bull.*, 1914, *xxv*, 205.
- (26) GUTHRIE: *Journ. Amer. Med. Assoc.*, 1917, *lxix*, 1394.
- (27) VAN SLYKE: *Journ. Biol. Chem.*, 1917, *xxx*, 347.
- (28) PETERS: *This Journal*, 1917, *xliv*, 84.
- (29) JANEWAY AND JACKSON: *Proc. Soc. Exper. Biol. and Med.*, 1915, *xii*, 193.
- (30) ERLANGER AND WOODYATT: *Journ. Amer. Med. Assoc.*, 1917, *lxix*, 1410.
- (31) ERLANGER, GESELL, GASSER AND ELLIOTT: *Journ. Amer. Med. Assoc.*, 1917, *lxix*, 2089.
- (32) MANN: *Journ. Amer. Med. Assoc.*, 1918, *lxxi*, 1184.
- (33) MORRIS: *Journ. Amer. Med. Assoc.*, 1917, *lxviii*, 1391.
- (34) BAYLISS: *Op. cit.*, 60.

PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE

III. THE PRODUCTION OF LIGHT BY *LUCIOLA VITTICOLLIS* IS AN OXIDATION¹

SAKYO KANDA

From the Marine Biological Laboratory, Kyushu Imperial University, Tsuyazaki (Fukuoka), Japan

Received for publication May 22, 1920

INTRODUCTION

The problem whether the production of light by a fire-fly is an oxidation is an old one, having been raised as long ago as 1783 (2, p. 355). Unfortunately, different investigators offer varying results. Spallanzani, for instance, found that the light produced by *Lampyris* disappeared in N_2 , H_2 and CO_2 , but it appeared again at the admission of air or, better still, O_2 . It is doubtful how exact his method was, because it was a work of 1796. On the other hand, according to Macartney, the *Lampyris* produced a brilliant light without O_2 , and it neither became stronger in O_2 nor weaker in H_2 (2, p. 356).

Mangold well points out, therefore, the status of this problem and states:

¹ In his paper in this Journal, January, 1920, Doctor Kanda stated that the production of light by *Cypridina* is not an oxidation. I think it will be admitted that his results were convincing to the extent of showing either that *no* oxygen was required or *very little* (as much as might, in spite of his elaborate precautions, have been present as an impurity). In the interest of clear discussion I believe it should be known that in a personal letter to me, Doctor Kanda now adopts the second alternative. The paper referred to is, I take it, to be interpreted as proving that the luminous material in *Cypridina* is very rapidly destroyed, without proportional light production, if any considerable amount of oxygen is present; and that the long-continued strong light production which he observed was due to the very small amount of oxygen present as an impurity in the gases used. As Doctor Kanda forwarded his manuscript for publication in English through me, I am venturing to add this note.—E. P. Lyon.

Die Frage nach der Bedeutung des Sauerstoffes für die Lumineszenz ist hier aber noch nicht . . . zu einem endgültigen und völlig klaren Abschluss gekommen, zumal noch die letzten Arbeiten über Leuchtkäfer zu scheinbar entgegengesetzten Ergebnissen geführt haben. Die Methodik spielt hier ja eine besonders grosse Rolle, zumal es für einwandfreie Versuche erforderlich ist, so geringe Mengen freien Sauerstoffes auszuschliessen . . . (2, p. 355).

In short, the problem of oxidation in question is by no means settled.

The writer, therefore, made an attempt to settle this problem with new methods and apparatus devised for "einwandfreie Versuche," as Mangold puts it; and he found that the results of these experiments were quite decisive. In this paper, therefore, he will report these results together with the description of methods and apparatus, which were new as far as he is aware. The work was carried out at the Science Department of the Kyushu Imperial University. The writer's thanks are due to Dr. Tsuneya Marusawa, the professor of physical chemistry, for his generous help and suggestions, and also to Mr. Tetsuzo Hagiwara, Doctor Marusawa's assistant, who assisted the writer all the way through the work. The writer appreciates Prof. Ayao Kuwaki's kindness for the privilege of the use of the laboratory.

MATERIAL

The material used for all the following experiments was a Japanese fire-fly, *Luciola vitticollis*. The luminous organ of this species differs according to the sex. The luminous organ of the male, which is smaller in size than the female, consists of the last two segments of its abdomen, while that of the latter consists of only one segment next to the last.

The luminous organs of the male which were used for all the following experiments, except one series, were carefully cut off from the rest of the body of the live animals. The luminous organs of the female were specially prepared for one series of experiments which were carried out to determine the quantity of oxygen to be consumed by the organs in the oxidation for the production of light. This will be mentioned later on.

THE PREPARATION OF PURE GASES

At first the writer used H_2 , N_2 and CO_2 gases which were prepared by ordinary methods and also O_2 from a bomb. The intensity of light produced by the luminous organs of the animals was always strongest

and lasted longest in oxygen. The production of light, however, also resulted, though only for a short time, when H_2 , N_2 and CO_2 gases were used. On the other hand, no light was produced in vacuum. These peculiar results led the writer to doubt whether the gases used were in reality pure, though they were prepared with special care. The careful analysis of H_2 and N_2 gases with an Orsat's apparatus revealed that they were impure. Their impurity extended even to 1-5 per cent, due to the mixture of O_2 . The writer has become con-

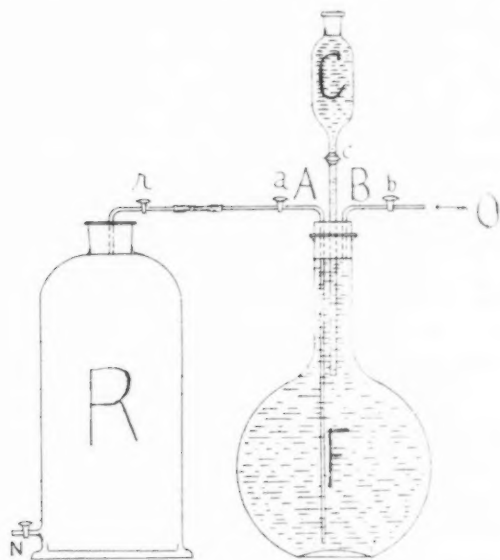


FIG. 1

vinced since then that gas washers which are used in an ordinary method of gas preparation could by no means purify a gas passed through them, though the air in them was evacuated beforehand.

An exact method, therefore, was to be planned to obviate the failures mentioned above. It was thought that if gas purified by an analytical method could be used, it would serve for this purpose. So a new gas holder was devised, as shown in figure 1. In the first place, the flask, *F*, was filled with a gas-absorbing solution, i.e., 150 cc. of

20 per cent $C_6H_3(OH)_3 + 800$ cc. of saturated KOH for O_2 , for example. The solution was drawn in the glass tubes, *A* and *B*, and a separating funnel, *C*, up to the stopcocks, *a*, *b* and *c*. The tube *B* was connected to one of the arms of the Orsat's apparatus by means of a rubber tube. All air in the rubber tube and in the rest of the glass tube *B*, from the stopcock *b*, was evacuated before the connection with the Orsat's was made. A receiving bottle, *R*, which was connected by means of a rubber tube to the glass tube *A* was evacuated and sealed up.

Now 100 cc. of a gas, either H_2 or N_2 , which was carefully prepared in the laboratory, were introduced into the burette of the Orsat's apparatus. Oxygen gas which was always mixed as an impurity with H_2 and N_2 gases was repeatedly absorbed by the alkaline pyrogallol solution in a pipette of the apparatus until the volume of the gas in the burette became constant. The gas thus purified was to be preserved in the flask *F* of figure 1. In order that the gas might be drawn into the flask, a stopcock of the arm of the Orsat's, say *O*, to which the flask was connected was to be opened. Thus the rubber tube in the vacuum was now filled with the gas. Then the stopcock *b* was opened. And lastly, the stopcock *a* was opened with careful regulation not to draw the solution in the bottle *R* from the flask *F* too fast. When about all the gas in the burette of the Orsat's was drawn out, all stopcocks just mentioned above were again closed. This same procedure was repeated several times until a desirable amount of the gas had filled the flask *F*. The virtue of this method was that if any traces of O_2 gas were left with the gas in the flask *F*, though hardly possible, the solution in the flask would absorb O_2 during the period of preservation. It is believed that gas absolutely free from even a trace of O_2 gas was available for use by this method.

The oxygen gas taken out from the bomb was also preserved in a flask with a saturated KOH solution in the same way as above after its analysis, although no trace of CO_2 was detected. It was found, however, that the O_2 gas from the bomb was impure to the extent of 2 to 3.25 per cent. Presumably the impurity was nitrogen. No attempt was made to remove the mixed gas or gases from O_2 , except CO_2 . Carbon dioxide was not used for the experiments of these series because of the difficulty of freeing it from O_2 .

METHOD

In the first place, ten isolated luminous organs of the male were placed in the experiment bottle, *E* (fig. 2). The bottle was fitted with a tight rubber stopper in which two glass tubes, *G* and *H*, with one stop-cock for each, were inserted. It was then fixed on an iron stand. The glass tube *G* was connected to the glass tube *A* and the glass tube *H* to one of the arms of a T-shaped glass tube, *J*, as illustrated in

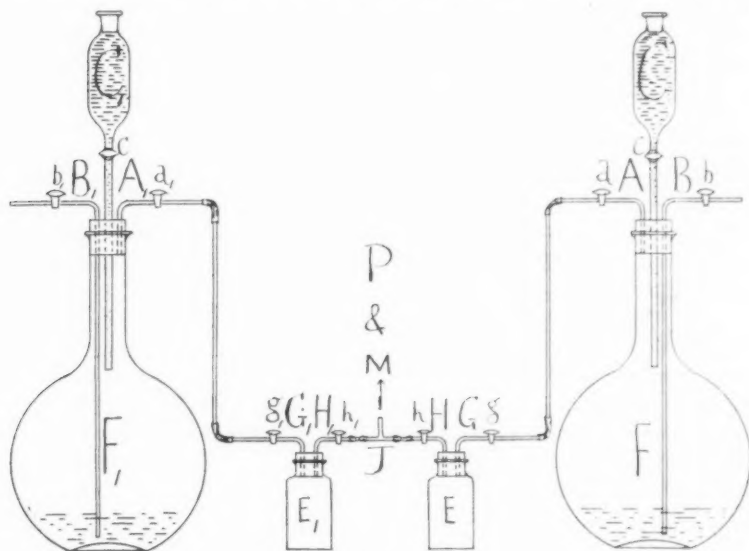


FIG. 2

figure 2. The experiment bottle, *E*₁, was also connected to the glass tube, *A*₁, of the flask, *F*₁, in the same way as just mentioned above. And one of the arms of the T-shaped glass tube *J* was connected to a Gaede's oil vacuum pump, *P*, through a manometer, *M*. As it was essential to exclude all air from without, melted paraffin was put all over the rubber stoppers of the experiment bottles *E* and *E*₁, especially those places where the stopper and glass were in contact.

The vacuum pump was then started to evacuate air in the experiment bottle *E* or *E*₁. Meanwhile the stopcocks *g* and *H* were opened. When the manometer reached the zero point, the stopcock *H* was closed

and the pump was stopped. Of course, some air was still to be left in the bottle and connections² because the pressure was not an absolute zero. Nevertheless, the material in the bottle produced no light. In order to make the vacuum of the experiment bottle and other spaces complete, the procedure of evacuation was repeated two or three times after filling the bottle with the pure gas which was to be used. For this purpose, the stopcocks *a* and *c*³ were opened and the bottle *E* was filled with the gas. After this filling was complete the stopcocks *a* and *c* were closed and the pump was again started to work. This procedure was repeated two or three times. After the last evacuation of the gas, the stopcocks *g* and *h* were closed, and the gas admitted up to the stopcock *g*, opening the stopcocks *a* and *c*. The last procedure of this experimentation was simply to introduce the gas into the bottle *E* to see whether any light was produced by the material or not. But this should be done together with the bottle *E*₁ which was prepared in the same way.

As O₂ gas was to be used in the bottle, *E*₁, it was thought that two complete evacuations might be enough. After the last evacuation, the stopcocks *g*₁ and *h*₁ were closed and the stopcocks *a*₁ and *c*₁ were opened. In so doing, the gas was admitted up to the stopcock *g*₁. Now both the bottles, *E* and *E*₁ were ready for the experiment. The room was then darkened. The stopcock *g*₁ with the left hand and the stopcock *g* with the right were opened at the same time. In this way, O₂ gas into the bottle *E*₁ and H₂ or N₂ gas, as the case may be, into the bottle *E*, were admitted at the same time. Thus the production of light by the isolated luminous organs of fire-flies in the two gases was observed simultaneously.

The O₂ experiment was always carried out in comparison with any other experiment as a control of special kind, besides a second control for which air was used. It was thought that the evacuation of air in the experiment bottle might have some effect on the production of light

² As the Gaede's pump is capable of developing a vacuum 0.001 mm. of Hg., the air left is only $\frac{1}{760,000}$ of one atmosphere after one evacuation. It may be said that there were originally about 12.6 cc. of O₂ in the experimental bottle of capacity of 60 cc., as the amount of O₂ in air is about 21 per cent. There is, therefore, only about 0.0000165 cc. of O₂ left after one evacuation.

³ The alkaline pyrogallol solution was always poured into the funnel, *C*, by means of a rubber tube connected to the stopcock, *N*, of the bottle, *R*, which was already disconnected from the flask, *F*.

as the result of taking water content away from the material or of some stimulation and other change. Besides the control using air, some experiments were performed by introducing air from a glass gas-holder into the experiment bottle, just the same as the other gases were introduced, after the evacuation of air. These experiments were also compared with those of O_2 , as well as with the controls in which air was used without any treatment. It should be remarked that the intensity of light produced by the material was much stronger in air which was introduced *after evacuation* than in air without any treatment. The writer will try to explain this fact later.

EXPERIMENTAL

As already stated, the purpose of this investigation was to determine whether the production of light by the fire-fly is an oxidation, as is generally assumed, or not. The results obtained prove what most previous authors believed. These experiments were, of course, repeated several times with no exception, when conditions were properly controlled. If there was any exception, it was found that either the gas used was impure, or the method or apparatus imperfect.

The production of light by the luminous organs of the fire-fly in varying gas atmosphere and condition: The methods of these experiments were very simple, as already described in the previous section. Table 1 is the summary of the results of these experiments. The figures in the table simply show a comparative intensity of light produced by the isolated luminous organs of the male in a given gas atmosphere. The figure "4", for example, means that the most intense light was produced by the material in one of the four gases, including air.

As table 1 has shown, no light was produced by the luminous organs in H_2 and N_2 atmospheres or in a vacuum. But the admission of air or better still, O_2 gas, resulted in a brilliant light. Fortunately, once the writer had N_2 gas in which 1 per cent of O_2 gas was mixed. He therefore tried to see whether the material would produce light or not. It was found then that the material produced intenser light in this mixture than in air. The light in the former continued for about 12 hours, while the control in air lasted for about 70 hours at about $20^\circ C$. About 3 hours after the extinction of light, the material produced light at the admission of air. These results will convince any unprejudiced minds that the production of light by the material is an oxidation. Furthermore, it is evident that free oxygen is absolutely neces-

sary for an oxidation of this sort, as the material produced no light in vacuum. And it seems therefore probable that even though some oxygen supplier or carrier is assumed to exist in the cells or tissues of the material, it plays no rôle by itself alone in this process of oxidation for the production of light.

The writer stated in the second paper of this series that the production of light by dried crushed Cypridinas was not an oxidation. Some Japanese critics thought that this statement was dogmatic beyond the facts actually found. Their reason was that the writer showed only that no oxygen in the medium was necessary for the production of light by the material, but he did not show at all that oxygen contained in the cells or tissues of the material was not used. The writer could make no answer to this objection. Now it may be answered

TABLE I

The production of light by the isolated luminous organs of Lucifolias in varying gas atmosphere and condition

GAS AND CONDITION	INTRODUCTION OF O ₂ AFTER EVACUATION	INTRODUCTION OF AIR AFTER EVACUATION	INTRODUCTION OF N ₂ + 1 PER CENT O ₂ AFTER EVACUATION	AIR CONTROL	INTRODUCTION OF H ₂ AFTER EVACUATION	INTRODUCTION OF N ₂ AFTER EVACUATION	VACUUM
Comparison of intensity of light.	4	3	2	1	0	0	0
Readmission of air	4	2	2 (?)	1	3	3	3

that it is not probable that any oxygen in the cells, or tissues of the dried crushed Cypridinas is used for the production of light by them, because no oxygen in the cells or tissues of the luminous organs of the fire-flies seems to be used for their production of light, even though this process is certainly an oxidation.

As already stated, the light produced by the material in air admitted after one evacuation was much stronger than that produced in air without any treatment. This fact could not be explained on a mere basis of the volume of oxygen contained in air. But as the cells and tissues of the material were alive and some nerve ganglia seemed to be located in the tissues, it seemed possible that stimulation by mechanical agitation occurred when air was admitted after an evacuation. This might be the same phenomenon as in the case of the fire-flies producing a stronger light when water is sprinkled on the cage contain-

ing them, or when the cage is shaken. Besides such biological factor, the surface of contact may also act. Evacuation may increase the contact-surface of the material for the readmitted oxygen gas and in consequence the rate of oxidation may increase. Whatever the reason, the result was a stronger light when air was readmitted after evacuation. The fact that the intensity of light produced by the material in the gaseous mixture of N_2 with 1 per cent of O_2 admitted after evacuation was stronger than that of light produced in air with no evacuation may also be explained in the same way. That the light produced by the material in air which contains about 21 per cent of O_2 is weaker than that in the mixture of N_2 with 1 per cent of O_2 is incomprehensible if considered merely from the viewpoint of an oxidation. But it is not necessarily so if the biological and physico-chemical factors, mechanical agitation and surface action just mentioned above, are considered.

An estimation of oxygen consumed by the luminous organs: An attempt was made to estimate the amount of O_2 converted into CO_2 in the production of light by the isolated luminous organs of the female. A preliminary experiment showed that quite a large amount of CO_2 was given off during the production of light by the luminous organs. This encouraged the writer to undertake further careful experiments. The method of this series of experiments was a little different from others, though quite simple. The bottle shown in figure 3 was used for this experimentation.

In the first place, the experiment material was isolated as carefully as possible to minimize the admission of other substances, which might in some way obscure the results. For this purpose the luminous organ of the female was more suitable than that of the male, because the female luminous organ of this species consists of only one abdominal segment next to the last, as already stated. If the thoracico-abdominal regions were pressed by the fingers of the left hand, the last two or three segments of the abdomen were stretched out. Then the last segment was carefully cut off by means of sharp seissors and the thoracico-abdominal regions were again pressed hard. In doing so, eggs and other matter contained in the abdomen were pressed out from the cut. They were all cleaned off with special care. After this cleaning, the luminous segments of sixty females thus isolated were placed in the experiment bottle *E* shown in figure 3. The bottle *E* was tightly fitted with a rubber stopper in which two capillary glass tubes, *A* and *B*, were inserted. The tube *B* was connected by



FIG. 3

means of a rubber tube to one of the arms of the Orsat's apparatus for gas analysis. The tube *A* which had one stopcock, *a*, was connected to the vacuum pump through a manometer. After all connections were thus made, melted paraffin was put all over the stopper of the bottle *E* as usual. Now the stopcock of the arm of the Orsat's, say *o*, to which the tube *B* was connected, was closed. The pump was started to evacuate the air in the bottle *E*. After complete evacuation, the stopcock *a* was closed.

Exactly 100 cc. of O_2 gas, which contained about 2.14 per cent N_2 but was absolutely free from CO_2 and was held in the burette of the Orsat's, were ready to be sent into the bottle *E* at any time. Now the stopcock *O* was opened. Oxygen gas was thus introduced into the bottle *E* from the burette. After this filling was over, the stopcock *O* was closed. The exact volume of the gas introduced into the bottle was read and the rest of the gas left in the burette was blown out. At the same time the barometer and temperature of the laboratory were read. For certain hours the material thus treated was allowed to use O_2 for the production of light, the exact volume of which was already known.

Twenty-five hours from the time of treatment the material was still producing light. But it was thought that further delay might complicate the results by increasing CO_2 gas which might be given off due to the decay of the material, or to other causes. Therefore the tube *A* was connected to a syphon by means of which distilled water was caused to run into the bottle *E*. The stopcocks *O* and *a* were opened in the order mentioned. The gas contained in the bottle was thus displaced by the water and returned to the burette. When the water reached the stopcock *Q*, the latter was closed. The volume of the returned gas and the temperature and barometer were read at the same time. The carbon dioxide mixed with the O_2 gas was absorbed by a concentrated KOH solution until the gas volume became constant. The O_2 was also absorbed by the alkaline pyrogallol solution after an addition to it of 16 cc. of N_2 . The results of the experiments calculated by reducing to the normal conditions are given in table 2.

The amount of O_2 consumed in the production of light by the material was found to be 6.01 cc., as shown in table 2. But the methods of the experiment were not beyond criticisms, which may be mentioned as follows: *a*, The gas in the burette of the Orsat's apparatus was displaced by distilled water. It was certain that the water in the burette would dissolve CO_2 which was sent for analysis from the experiment

bottle into the burette, after the luminous organs had used O_2 for the production of light. An error would thus be introduced into the results. In other experiments, therefore, the water in question was replaced by mercury before the gaseous mixture of O_2 and CO_2 was sent back into the burette. Unfortunately, however, a certain technical fault was found in the process of analysis. So these results have been ignored and the experiments made with water displacement are published. *b*, It was not at all certain whether the whole amount of O_2 , i.e., 6.01 cc., was exclusively used up for the production of light by the isolated luminous segments, as some of it might have been used for oxidation of substances of the segments independent of the process of the light production.

The writer was anxious to remove as far as possible these errors and objections just mentioned. It was, however, late in the season and the material could not longer be secured. He therefore reports the result as it was, though unfinished.

TABLE 2

An estimation of O_2 consumed by the isolated luminous organs of Luciolas for 25 hours

O_2 ORIGINALLY USED	$O_2 + CO_2$ AFTER 25 HOURS	DEVELOPED CO_2 FOUND	O_2 REMAINING	CONSUMED O_2 FOUND
cc.	cc.	cc.	cc.	cc.
58.52	58.17	5.66	52.51	6.01

A relation of the production of light to water: It was supposed that if the production of light by the material was an oxidation, the material might be preserved longer in vacuum than in air. So the following experiments were tried. Seventeen glass tubes of the capacity of about 25 cc. were drawn narrow at one end and sealed up at the other end. Isolated luminous organs of ten males were placed in each tube. Three of these tubes were sealed up at a certain time. These were the controls of one kind. Another three tubes which were thoroughly evacuated once were sealed up after readmitting air. These were controls of another kind. The material in the controls of these two kinds was of course producing light. The rest of the tubes were sealed up while the process of evacuation was going on. The material in these tubes was not producing light. At an interval of 2 or 3 hours, one of these tubes was opened in the dark room to see whether the material produced light or not. In doing so it was thought that the

exact time might be detected at which the material no longer produced light. But as table 3 has shown, the results were contradictory and were quite contrary to the writer's expectation. That is to say, the material was preserved longer in the air than in the vacuum.

The material of the controls of the second kind which were evacuated and were sealed up after admission of air, seemed to furnish an explanation to the riddle. There should be no difference in the durability of light between the first and second controls because the volume of air in both was practically equal. But perhaps the material which was temporarily exposed to a vacuum might have lost some water during the process. The loss of water might perhaps have shortened the

TABLE 3

The production of light by the isolated luminous organs in air admitted after the enclosure in vacuum

CONDITION TIME IN HOURS	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN AIR	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN AIR AFTER EVACUATION	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN VACUUM	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN VACUUM AT THE ADMISSION OF AIR
1	+	+	-	+
5	+	+	-	+
10	+	+	-	+
15	+	+	-	+
20	+	+	-	+
25	+	+	-	+
30	+	+	-	-
35	+	+	-	-
40	+	-	-	-
45	+	-	-	-
50	-	-	-	-

endurance of the light-producing substance. This view may also explain the failure of experiments in which all the material was sealed up for a long time in the vacuum tubes. It may be asserted as probable, therefore, that water is necessary for the production of light by the fire-fly.

This view is strengthened if the following fact is considered. That is to say, dried crushed luminous organs of the fire-fly produce light, though faint, if moistened. The fire-fly seems, however, to be quite different from Cypridinas. The more dried the longer the latter is preserved, while the dried fire-fly or its luminous organ is preserved only 5 or 6 days. That is to say, the dried luminous organ of the

fire-fly did not produce light after 5 or 6 days even though moistened. A question arose whether the luminous organs of fire-flies produced light when they were dead if moistened or not. And absolute dryness of the organs in question might be one of the causes of death. This idea was tested but no decisive results were obtained.

The effect of temperature: Harvey states that "*Luciola photogenin* is destroyed at about 42° , while the photophelein is still active after ten minutes boiling" (1, p. 348). The writer found that the light produced by the isolated luminous organs of *Luciola vitticollis* disappeared when heated at 50°C ., but it returned again when cooled. The return of light took place after about 5 or 10 minutes and it was very faint.

SUMMARY AND CONCLUSION

1. The material used for experiments was a Japanese fire-fly, *Luciola vitticollis*.

2. The gases used for experiments were H_2 , N_2 and O_2 .

3. New methods and apparatus were contrived to purify and manipulate the gases to fit the purposes of this investigation.

4. The isolated luminous organs of the animals produced no light in H_2 and N_2 or in vacuum. The oxygen of the cells or tissues of the organs, therefore, seemed not to be used for the production of light.

5. The intensity of light produced by the isolated luminous organs was greatest in O_2 atmosphere, next in air which was introduced after evacuation, then in N_2 mixed with 1 per cent of O_2 and last in air.

6. The isolated luminous organs of sixty females which were placed in 58.52 cc. of O_2 gave off 5.66 cc. CO_2 in 25 hours. The amount of O_2 consumed was 6.01 cc.

7. Water seemed to be necessary for the production of light by the isolated luminous organs.

8. The light produced by the isolated luminous organs disappeared when heated to 50°C ., but it appeared again when cooled.

The principal conclusion on the basis of the experimental results mentioned above is that the production of light by *Luciola vitticollis* is an oxidation.

BIBLIOGRAPHY

- (1) HARVEY: This Journal, 1917, xlii, 318.
- (2) MANGOLD: Winterstein's Handb. verg. Physiol., 1910, iii, 225.